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(54) Title: MODULATORS OF MOLECULES WITH PHOSPHOTYROSINE RECOGNITION UNITS (57) Abstract <p>The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to their use for purification of proteins or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including protein tyrosine phosphatases (PTPases) and protein with Src-homology-2 domains, in <i>in vitro</i> systems, micro-organisms, eukaryotic cells, whole animals and human beings. The novel organic compounds are compounds of the general formula (I) $(L)_n-Ar_1-R_1-A$, wherein $(L)_n$, n, Ar_1, R_1 and A are defined as defined in the application.</p>		

MODULATORS OF MOLECULES WITH PHOSPHOTYROSINE RECOGNITION UNITS

BACKGROUND OF THE INVENTION

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Field of the Invention

The present invention relates to novel organic compounds, to methods for their preparation, to compositions containing them, to their use for treatment of human and animal disorders, to
10 their use for purification of proteins or glycoproteins, and to their use in diagnosis. The invention relates to modulation of the activity of molecules with phospho-tyrosine recognition units, including protein tyrosine phosphatases (PTPases) and proteins with Src-homology-2 domains, in *in vitro* systems, micro-organisms, eukaryotic cells, whole animals and human beings.

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Background of the invention

Phosphorylation of proteins is a fundamental mechanism for regulation of many cellular processes. Although protein phosphorylation at serine and threonine residues is
20 quantitatively dominating in eukaryotic cells, reversible tyrosine phosphorylation seems to play a pivotal role in regulation of cell growth and differentiation as well as in neoplastic transformation (Hunter, *Cell* 80: 225-236 (1995); Schlessinger and Ullrich, *Neuron* 9: 383-391 (1992); Cantley et al., *Cell* 64: 281-302 (1991); Ullrich and Schlessinger, *Cell* 61:203-212 (1990); Hunter, *Curr. Opin. Cell. Biol.* 1: 1168-1181 (1989)); Hunter and Cooper, *Annu.*
25 *Rev. Biochem.* 54: 897-930 (1985)).

The regulation of protein tyrosine phosphorylation *in vivo* is mediated by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). The level of protein tyrosine phosphorylation of cellular proteins is determined by the
30 balanced activities of PTKs and PTPase (Hunter, 1995, *supra*).

PTPases - an overview

The protein phosphatases are composed of at least two separate and distinct families
5 (Hunter, T., *Cell* 58: 1013-1016 (1989)) the protein serine/threonine phosphatases and the PTPases.

The PTPases are a family of enzymes that can be classified into two groups: a) intracellular
or nontransmembrane PTPases and b) receptor-type or transmembrane PTPases.

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Intracellular PTPases: All known intracellular type PTPases contain a single conserved catalytic phosphatase domain consisting of 220-240 amino acid residues. The regions outside the PTPase domains are believed to play important roles in localizing the intracellular PTPases subcellularly (Mauro, L.J. and Dixon, J.E. *TIBS* 19: 151-155 (1994)). The first
15 intracellular PTPase to be purified and characterized was PTP1B which was isolated from human placenta (Tonks *et al.*, *J. Biol. Chem.* 263: 6722-6730 (1988)). Shortly after, PTP1B was cloned (Charbonneau *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 5252-5256 (1989); Chernoff *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 2735-2789 (1989)). Other examples of intracellular PTPases include (1) T-cell PTPase (Cool *et al.* *Proc. Natl. Acad. Sci. USA* 86: 5257-5261
20 (1989)), (2) rat brain PTPase (Guan *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 1501-1502 (1990)), (3) neuronal phosphatase STEP (Lombroso *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 7242-7246 (1991)), (4) ezrin-domain containing PTPases: PTPMEG1 (Guet *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 5867-57871 (1991)), PTPH1 Yang and Tonks, *Proc. Natl. Acad. Sci. USA* 88: 5949-5953 (1991), PTPD1 and PTPD2 (Møller *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 7477-7481 (1994)), FAP-1/BAS (Sato *et al.*, *Science* 268: 411-415 (1995); Banville *et al.*, *J. Biol. Chem.* 269: 22320-22327 (1994); Maekawa *et al.*, *FEBS Letters* 337: 200-206 (1994)), and SH2 domain containing PTPases: PTP1C/SH-PTP1 (Plutsky *et al.*, *Proc. Natl. Acad. Sci. USA* 89: 1123-1127 (1992); Shen *et al.*, *Nature Lond.* 352: 736-739 (1991)) and PTP1D/Syp/SH-PTP2 (Vogel *et al.*, *Science* 259: 1611-1614 (1993); Feng *et al.*, *Science*
25 259: 1607-1611 (1993); Bastein *et al.*, *Biochem. Biophys. Res. Comm.* 196: 124-133 (1993)).

Low molecular weight phosphotyrosine-protein phosphatase (LMW-PTPase) shows very little sequence identity to the intracellular PTPases described above. However, this enzyme belongs to the PTPase family due to the following characteristics: (i) it possesses the PTPase active site motif: Cys-Xxx-Xxx-Xxx-Xxx-Arg (Ciri *et al.*, *Eur. J. Biochem.* 214: 647-657 (1993)); (ii) this Cys residue forms a phospho-intermediate during the catalytic reaction similar to the situation with 'classical' PTPases (Ciri *et al.*, *supra*; Chiarugi *et al.*, *FEBS Lett.* 310: 9-12 (1992)); (iii) the overall folding of the molecule shows a surprising degree of similarity to that of PTP1B and *Yersinia* PTP (Su *et al.*, *Nature* 370: 575-578 (1994)).

Receptor-type PTPases consist of a) a putative ligand-binding extracellular domain, b) a transmembrane segment, and c) an intracellular catalytic region. The structures and sizes of the putative ligand-binding extracellular domains of receptor-type PTPases are quite divergent. In contrast, the intracellular catalytic regions of receptor-type PTPases are very homologous to each other and to the intracellular PTPases. Most receptor-type PTPases have two tandemly duplicated catalytic PTPase domains.

The first receptor-type PTPases to be identified were (1) CD45/LCA (Ralph, S.J., *EMBO J.* 6: 1251-1257 (1987)) and (2) LAR (Streuli *et al.*, *J. Exp. Med.* 168: 1523-1530 (1988)) that were recognized to belong to this class of enzymes based on homology to PTP1B (Charbonneau *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 5252-5256 (1989)). CD45 is a family of high molecular weight glycoproteins and is one of the most abundant leukocyte cell surface glycoproteins and appears to be exclusively expressed upon cells of the hematopoietic system (Trowbridge and Thomas, *Ann. Rev. Immunol.* 12: 85-116 (1994)).

The identification of CD45 and LAR as members of the PTPase family was quickly followed by identification and cloning of several different members of the receptor-type PTPase group. Thus, 5 different PTPases, (3) PTP α , (4) PTP β , (5) PTP δ , (6) PTP ϵ , and (7) PTP ζ , were identified in one early study (Krueger *et al.*, *EMBO J.* 9: 3241-3252 (1990)). Other examples of receptor-type PTPases include (8) PTP γ (Bamea *et al.*, *Mol. Cell. Biol.* 13:

1497-1506 (1995)) which, like PTP ζ (Knueger and Saito, *Proc. Natl. Acad. Sci. USA* 89: 7417-7421 (1992)) contains a carbonic anhydrase-like domain in the extracellular region, (9) PTP μ (Gebbink *et al.*, *FEBS Letters* 290: 123-130 (1991), (10) PTP κ (Jiang *et al.*, *Mol. Cell. Biol.* 13: 2942-2951 (1993)). Based on structural differences the receptor-type PTPases may be classified into subtypes (Fischer *et al.*, *Science* 253: 401-406 (1991)): (I) CD45; (II) LAR, PTP δ , (11) PTP σ ; (III) PTP β , (12) SAP-1 (Matozaki *et al.*, *J. Biol. Chem.* 269: 2075-2081 (1994)), (13) PTP-U2/GLEPP1 (Seimiya *et al.*, *Oncogene* 10: 1731-1738 (1995); (Thomas *et al.*, *J. Biol. Chem.* 269: 19953-19962 (1994)), and (14) DEP-1; (IV) PTP α , PTP ϵ . All receptor-type PTPases except Type IV contain two PTPase domains. Novel PTPases are continuously identified, and it is anticipated that more than 500 different species will be found in the human genome, i.e. close to the predicted size of the protein tyrosine kinase superfamily (Hanks and Hunter, *FASEB J.* 9: 576-596 (1995)).

PTPases are the biological counterparts to protein tyrosine kinases (PTKs). Therefore, one important function of PTPases is to control, down-regulate, the activity of PTKs. However, a more complex picture of the function of PTPases now emerges. Several studies have shown that some PTPases may actually act as positive mediators of cellular signalling. As an example, the SH2 domain-containing PTP1D seems to act as a positive mediator in insulin-stimulated Ras activation (Noguchi *et al.*, *Mol. Cell. Biol.* 14: 6674-6682 (1994)) and of growth factor-induced mitogenic signal transduction (Xiao *et al.*, *J. Biol. Chem.* 269: 21244-21248 (1994)), whereas the homologous PTP1C seems to act as a negative regulator of growth factor-stimulated proliferation (Bignon and Siminovitch, *Clin. Immunol. Immunopathol.* 73: 168-179 (1994)). Another example of PTPases as positive regulators has been provided by studies designed to define the activation of the Src-family of tyrosine kinases. In particular, several lines of evidence indicate that CD45 is positively regulating the activation of hematopoietic cells, possibly through dephosphorylation of the C-terminal tyrosine of Fyn and Lck (Chan *et al.*, *Annu. Rev. Immunol.* 12: 555-592 (1994)).

Dual specificity protein tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphotyrosine as well as from phosphor-serine/threonine. dsPTPases contain the signature sequence of PTPases: His-

Cys-Xxx-Xxx-Gly-Xxx-Xxx-Arg. At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated protein kinase (MAPK): MAPK phosphatase (CL100, 3CH134) (Charles *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 5292-5296 (1993)); PAC-1 (Ward *et al.*, *Nature* 367: 651-654 (1994)); rVH6 (Mourey *et al.*, *J. Biol. Chem.* 271: 3795-3802 (1996)). Transcription of dsPTPases are induced by different stimuli, e.g. oxidative stress or heat shock (Ishibashi *et al.*, *J. Biol. Chem.* 269: 29897-29902 (1994); Keyse and Emslie, *Nature* 359: 644-647 (1992)). Further, they may be involved in regulation of the cell cycle: cdc25 (Millar and Russell, *Cell* 68: 407-410 (1992)); KAP (Hannon *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 1731-1735 (1994)). Interestingly, tyrosine dephosphorylation of cdc2 by a dual specific phosphatase, cdc25, is required for induction of mitosis in yeast (review by Walton and Dixon, *Annu. Rev. Biochem.* 62: 101-120 (1993)).

PTPase specificity

Several studies have addressed the question of PTPase specificity using synthetic peptides and provided important insight with respect to primary structural sequence requirements for substrate recognition (Ramachandran *et al.*, *Biochemistry* 31: 4232-4238 (1992); Cho, H. *et al.*, *Biochemistry* 31: 133-138 (1992); Zhang, Z.-Y. *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 4446-4450 (1993); Zhang, Z.-Y. *et al.*, *Biochemistry* 33: 2285-2290 (1994)). However, an obvious limitation of this approach is the lack of defined three-dimensional structure of the peptide analogs. Likewise, the PTPases utilized for these analyses are removed from their natural environment. Since at least part of the PTPase specificity seems to be conveyed by a defined subcellular localization (Mauro and Dixon, *TIBS* 19: 151-155 (1994)), it is essential that such studies are complemented with measurements of PTPase activity towards cellular substrates in intact cells.

Phosphotyrosine recognition in signal transduction

Hormones, growth factors, cytokines, antigens, extracellular matrix components as well as molecules positioned at the cell surface induce signal transduction by binding to specific cell surface structures or receptors on target cells (reviewed in Pawson, *Nature* 373: 573-580

(1995)). The resulting cellular signal is often mediated through a series of phosphorylation and dephosphorylation reactions on tyrosine residues of signalling molecules. To allow efficient and selective signalling, several recognition units for phosphotyrosine (pTyr) have developed during evolution: a) PTPases; b) Src-homology-2 (SH2) domains; c) pTyr-binding (PTB) domains. As described above, the recognition of pTyr by PTPases leads to dephosphorylation with concomitant dissociation from the molecular target. Dephosphorylation may either lead to upregulation or downregulation of the signal. In contrast, SH2 domains and PTB domains primarily act as docking molecules with little or no catalytic activity. In other words, tyrosine phosphorylated proteins have the capacity to bind other proteins containing SH2 domains or PTB domains thereby controlling the subcellular location of signalling molecules. There appears to be a significant degree of selectivity in SH2 domain recognition of pTyr and their surroundings. Thus, SH2 domains from the Src kinase family bind the peptide pTyr-Glu-Glu-Ile in a relatively selective manner, whereas the PTPD1 seems to recognize at least five, primarily hydrophobic residues C-terminal to the pTyr (Pawson, *supra*). Certain PTPase domains, in particular the C-terminal domain of some receptor-type PTPases, seem to have little or no catalytic activity. It may be hypothesized that these domains have a function as pTyr recognition units similar to SH2 domains and PTB domains. Inhibition of signal transduction processes could, in principle, be achieved by using non-hydrolyzable pTyr-containing peptides with preferential affinity for specific PTPases, SH2 domains or PTB domains. However, due to the lack of efficient bioavailability of peptides there is a need for development of either peptidomimetics or novel small molecules with preferential binding to pTyr recognition units of specific cellular targets. Such selective compounds can either initiate, increase or decrease defined signal transduction processes.

PTPases: Inhibitors

In an early study, vanadate was found to inhibit protein-tyrosine phosphatases in mammalian cells with a concomitant increase in the level of phosphotyrosine in cellular proteins leading to transformation (Klarlund, *Cell* 41: 707-717 (1985)). Vanadium-based phosphatase inhibitors are relatively unspecific. Therefore, to assess the importance of specific structures on PTPase activity more selective inhibitors are needed. One possibility for obtaining

selective PTPase inhibitors would be through design of different ancillary ligands for peroxovanadium-based compounds (Posner et al., *J. Biol. Chem.* 269: 4596-4604 (1994)). Another avenue taken by several investigators has been to incorporate nonhydrolyzable tyrosine phosphate analogs into specific peptide substrates: (1) phosphonomethyl phenylalanine (Zhang et al., *Biochemistry* 33: 2285-2290 (1994)); (2) difluorophosphoromethyl phenylalanine Burk et al., *Synthesis* 11: 1019-1020 (1991)); (3) L-O-malonyltyrosine (Kole et al., *Biochem. Biophys. Res. Commun.* 209: 817-822 (1995)); (4) cinnamic acid (Moran et al., *J. Am. Chem. Soc.* 117: 10787-10788 (1995); Cao et al., *Bioorganic Med. Chem. Lett.* 5: 2953-2958 (1995)); (5) sulfotyrosyl (Liotta et al., *J. Biol. Chem.* 269: 22996-23001 (1994)). A surprising degree of selectivity is observed with simple peptide analogs containing phosphonodifluoromethyl phenylalanine as a substitute for tyrosine (Chen et al., *Biochem. Biophys. Res. Commun.* 216: 976-984 (1995)). Important information has further been obtained with synthetic peptides containing sulfotyrosyl residues. A synthetic peptide corresponding to the amino acid sequence of a defined loop of the insulin receptor tyrosine kinase, Thr-Arg-Asp-Ile-Xxx-Glu-Thr-Asp-Xxx-Xxx-Arg-Lys (where Xxx denotes sulfotyrosyl), acts as a PTPase inhibitor (Liotta et al., 1994, *supra*). More importantly, this peptide, when tagged with stearic acid can penetrate cells, and stimulate the action of insulin (Liotta et al., 1994, *supra*).

20 PTPases: the insulin receptor signalling pathway/diabetes

Insulin is an important regulator of different metabolic processes and plays a key role in the control of blood glucose. Defects related to its synthesis or signalling lead to diabetes mellitus. Binding of insulin to its receptor causes rapid (auto)phosphorylation of several tyrosine residues in the intracellular part of the β -subunit. Three closely positioned tyrosine residues (the tyrosine-1150 domain) must all be phosphorylated to obtain full activity of the insulin receptor tyrosine kinase (IRTK) which transmits the signal further downstream by tyrosine phosphorylation of other cellular substrates, including insulin receptor substrate-1 (IRS-1) (Wilden et al., *J. Biol. Chem.* 267: 16660-16668 (1992); Myers and White, *Diabetes* 42: 643-650 (1993); Lee and Pilch, *Am. J. Physiol.* 266: C319-C334 (1994); White et al., *J. Biol. Chem.* 263: 2969-2980 (1988)). The structural basis for the function of the tyrosine-triplet has been provided by recent X-ray crystallographic studies of IRTK that showed

tyrosine-1150 to be autoinhibitory in its unphosphorylated state (Hubbard *et al.*, *Nature* 372: 746-754 (1994)).

Several studies clearly indicate that the activity of the auto-phosphorylated IRTK can be reversed by dephosphorylation *in vitro* (reviewed in Goldstein, *Receptor* 3: 1-15 (1993); Mooney and Anderson, *J. Biol. Chem.* 264: 6850-6857 (1989)), with the tri-phosphorylated tyrosine-1150 domain being the most sensitive target for protein-tyrosine phosphatases (PTPases) as compared to the di- and mono- phosphorylated forms (King *et al.*, *Biochem. J.* 275: 413-418 (1991)). It is, therefore, tempting to speculate that this tyrosine-triplet functions as a control switch of IRTK activity. Indeed, the IRTK appears to be tightly regulated by PTP-mediated dephosphorylation *in vivo* (Khan *et al.*, *J. Biol. Chem.* 264: 12931-12940 (1989); Faure *et al.*, *J. Biol. Chem.* 267: 11215-11221 (1992); Rothenberg *et al.*, *J. Biol. Chem.* 266: 8302-8311 (1991)). The intimate coupling of PTPases to the insulin signalling pathway is further evidenced by the finding that insulin differentially regulates PTPase activity in rat hepatoma cells (Meyerovitch *et al.*, *Biochemistry* 31: 10338-10344 (1992)) and in livers from alloxan diabetic rats (Boylan *et al.*, *J. Clin. Invest.* 90: 174-179 (1992)).

Relatively little is known about the identity of the PTPases involved in IRTK regulation. However, the existence of PTPases with activity towards the insulin receptor can be demonstrated as indicated above. Further, when the strong PTPase-inhibitor pervanadate is added to whole cells an almost full insulin response can be obtained in adipocytes (Fantus *et al.*, *Biochemistry* 28: 8864-8871 (1989); Eriksson *et al.*, *Diabetologia* 39: 235-242 (1995)) and skeletal muscle (Leighton *et al.*, *Biochem. J.* 276: 289-292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds act as potent hypoglycemic compounds *in vivo* (Posner *et al.*, *supra*). Two of these compounds were demonstrated to be more potent inhibitors of dephosphorylation of the insulin receptor than of the EGF-receptor.

It was recently found that the ubiquitously expressed SH2 domain containing PTPase, PTP1D (Vogel *et al.*, 1993, *supra*), associates with and dephosphorylates IRS-1, but apparently not the IR itself (Kuhné *et al.*, *J. Biol. Chem.* 268: 11479-11481 (1993); (Kuhné *et al.*, *J. Biol. Chem.* 269: 15833-15837 (1994)).

Previous studies suggest that the PTPases responsible for IRTK regulation belong to the class of membrane-associated (Faure *et al.*, *J. Biol. Chem.* 267: 11215-11221 (1992)) and glycosylated molecules (Häring *et al.*, *Biochemistry* 23: 3298-3306 (1984); Sale, *Adv. Prot. Phosphatases* 6: 159-186 (1991)). Hashimoto *et al.* have proposed that LAR might play a
5 role in the physiological regulation of insulin receptors in intact cells (Hashimoto *et al.*, *J. Biol. Chem.* 267: 13811-13814 (1992)). Their conclusion was reached by comparing the rate of dephosphorylation/inactivation of purified IR using recombinant PTP1B as well as the cytoplasmic domains of LAR and PTP α . Antisense inhibition was recently used to study the effect of LAR on insulin signalling in a rat hepatoma cell line (Kulas *et al.*, *J. Biol. Chem.* 270:
10 2435-2438 (1995)). A suppression of LAR protein levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced auto-phosphorylation. However, only a modest 35 percent increase in IRTK activity was observed, whereas the insulin-dependent phosphatidylinositol 3-kinase (PI 3-kinase) activity was significantly increased by 350 percent. Reduced LAR levels did not alter the basal level of IRTK tyrosine
15 phosphorylation or activity. The authors speculate that LAR could specifically dephosphorylate tyrosine residues that are critical for PI 3-kinase activation either on the insulin receptor itself or on a downstream substrate.

While previous reports indicate a role of PTP α in signal transduction through src activation
20 (Zheng *et al.*, *Nature* 359: 336-339 (1992); den Hertog *et al.*, *EMBO J.* 12: 3789-3798 (1993)) and interaction with GRB-2 (den Hertog *et al.*, *EMBO J.* 13: 3020-3032 (1994); Su *et al.*, *J. Biol. Chem.* 269: 18731-18734 (1994)), a recent study suggests a function for this phosphatase and its close relative PTP ϵ as negative regulators of the insulin receptor signal (Møller *et al.*, 1995 *supra*). This study also indicates that receptor-like PTPases play a
25 significant role in regulating the IRTK, whereas intracellular PTPases seem to have little, if any, activity towards the insulin receptor. While it appears that the target of the negative regulatory activity of PTPases α and ϵ is the receptor itself, the downmodulating effect of the intracellular TC-PTP seems to be due to a downstream function in the IR-activated signal. Although PTP1B and TC-PTP are closely related, PTP1B had only little influence on the
30 phosphorylation pattern of insulin-treated cells. Both PTPases have distinct structural features that determine their subcellular localization and thereby their access to defined

cellular substrates (Frangione *et al.*, *Cell* 68: 545-560 (1992); Faure and Posner, *Glia* 9: 311-314 (1993)). Therefore, the lack of activity of PTP1B and TC-PTP towards the IRTK may, at least in part, be explained by the fact that they do not co-localize with the activated insulin receptor. In support of this view, PTP1B and TC-PTP have been excluded as candidates for the IR-associated PTPases in hepatocytes based on subcellular localization studies (Faure *et al.*, *J. Biol. Chem.* 267: 11215-11221 (1992)).

The transmembrane PTPase CD45, which is believed to be hematopoietic cell-specific, was in a recent study found to negatively regulate the insulin receptor tyrosine kinase in the human multiple myeloma cell line U266 (Kulas *et al.*, *J. Biol. Chem.* 271: 755-760 (1996)).

PTPases: somatostatin

Somatostatin inhibits several biological functions including cellular proliferation (Lamberts *et al.*, *Molec. Endocrinol.* 8: 1289-1297 (1994)). While part of the antiproliferative activities of somatostatin are secondary to its inhibition of hormone and growth factor secretion (e.g. growth hormone and epidermal growth factor), other antiproliferative effects of somatostatin are due to a direct effect on the target cells. As an example, somatostatin analogs inhibit the growth of pancreatic cancer presumably via stimulation of a single PTPase, or a subset of PTPases, rather than a general activation of PTPase levels in the cells (Liebow *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2003-2007 (1989); Colas *et al.*, *Eur. J. Biochem.* 207: 1017-1024 (1992)). In a recent study it was found that somatostatin stimulation of somatostatin receptors SSTR1, but not SSTR2, stably expressed in CHO-K1 cells can stimulate PTPase activity and that this stimulation is pertussis toxin-sensitive. Whether the inhibitory effect of somatostatin on hormone and growth factor secretion is caused by a similar stimulation of PTPase activity in hormone producing cells remains to be determined.

PTPases: the immune system/autoimmunity

Several studies suggest that the receptor-type PTPase CD45 plays a critical role not only for initiation of T cell activation, but also for maintaining the T cell receptor-mediated signalling cascade. These studies are reviewed in: Weiss A., *Ann. Rev. Genet.* 25: 487-510 (1991);

Chan *et al.*, *Annu. Rev. Immunol.* 12: 555-592 (1994); Trowbridge and Thomas, *Annu. Rev. Immunol.* 12: 85-116 (1994).

The exact function of CD45 in lymphocyte activation is currently under intense investigation in many laboratories. Several studies suggest that the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family protein-tyrosine kinase (Mustelin *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 6302-6306 (1989); Ostergaard *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 8959-8963 (1989)). These authors hypothesized that the phosphatase activity of CD45 activates Lck by dephosphorylation of a C-terminal tyrosine residue, which may, in turn, be related to T-cell activation. In a recent study it was found that recombinant p56^{lck} specifically associates with recombinant CD45 cytoplasmic domain protein, but not to the cytoplasmic domain of the related PTP α (Ng *et al.*, *J. Biol. Chem.* 271: 1295-1300 (1996)). The p56^{lck}-CD45 interaction seems to be mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family protein-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri *et al.*, *J. Biol. Chem.* 270: 27987-27990 (1995)).

HePTP, a hematopoietic cell specific PTPase, is induced after activation of resting T cells and may play a role in late T cell activation or as a negative regulator of T cell responses (Zanke *et al.*, *Eur. J. Immunol.* 22: 235-239 (1992)). Likewise, the hematopoietic cell specific PTP1C seems to act as a negative regulator and play an essential role in immune cell development. In accordance with the above-mentioned important function of CD45, HePTP and PTP1C, selective PTPase inhibitors may be attractive drug candidates both as immunosuppressors and as immunostimulants. One recent study illustrates the potential of PTPase inhibitors as immunomodulators by demonstrating the capacity of the vanadium-based PTPase inhibitor, BMLOV, to induce apparent B cell selective apoptosis compared to T cells (Schieven *et al.*, *J. Biol. Chem.* 270: 20824-20831 (1995)).

PTPases: cell-cell interactions/cancer

Focal adhesion plaques, an *in vitro* phenomenon in which specific contact points are formed when fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal adhesion proteins are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, *Neuron* 11, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these proteins can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several intracellular PTPases with ezrin-like N-terminal domains, e.g. PTPMEG1 (Gu *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 5867-5871 (1991)), PTPH1 (Yang and Tonks, *Proc. Natl. Acad. Sci. USA* 88: 5949-5953 (1991)) and PTPD1 (Møller *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 7477-7481 (1994)). The ezrin-like domain show similarity to several proteins that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated by and associated with c-src *in vitro* and is hypothesized to be involved in the regulation of phosphorylation of focal adhesions (Møller *et al.*, *supra*).

PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion proteins, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 7280-7284 (1990)), can inhibit the transforming activity of v-erb and v-fms (Lammers *et al.*, *J. Biol. Chem.* 268: 22456-22462 (1993); Zander *et al.*, *Oncogene* 8: 1175-1182 (1993)). Moreover, it was found that transformation by the oncogenic form of the *HER2/neu* gene was suppressed in NIH 3T3 fibroblasts overexpressing PTP1B (Brown-Shimer *et al.*, *Cancer Res.* 52: 478-482 (1992)).

The expression level of PTP1B was found to be increased in a mammary cell line transformed with *neu* (Zhay *et al.*, *Cancer Res.* 53: 2272-2278 (1993)). The intimate relationship between tyrosine kinases and PTPases in the development of cancer is further evidenced by the recent finding that PTP ϵ is highly expressed in murine mammary tumors in transgenic mice over-expressing c-*neu* and v-Ha-*ras*, but not c-*myc* or *int-2* (Elson and

Leder, *J. Biol. Chem.* 270: 26116-26122 (1995)). Further, the human gene encoding PTP γ was mapped to 3p21, a chromosomal region which is frequently deleted in renal and lung carcinomas (LaForgia *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 5036-5040 (1991)).

5 In this context, it seems significant that PTPases appear to be involved in controlling the growth of fibroblasts. In a recent study it was found that Swiss 3T3 cells harvested at high density contain a membrane-associated PTPase whose activity on an average is 8-fold higher than that of cells harvested at low or medium density (Pallen and Tong, *Proc. Natl. Acad. Sci. USA* 88: 6996-7000 (1991)). It was hypothesized by the authors that density-dependent inhibition of cell growth involves the regulated elevation of the activity of the PTPase(s) in question. In accordance with this view, a novel membrane-bound, receptor-type PTPase, DEP-1, showed enhanced (≥ 10 -fold) expression levels with increasing cell density of WI-38 human embryonic lung fibroblasts and in the AG1518 fibroblast cell line (Östman *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 9680-9684 (1994)).

15 Two closely related receptor-type PTPases, PTP κ and PTP μ , can mediate homophilic cell-cell interaction when expressed in non-adherent insect cells, suggesting that these PTPases might have a normal physiological function in cell-to-cell signalling (Gebbink *et al.*, *J. Biol. Chem.* 268: 16101-16104 (1993); Brady-Kalnay *et al.*, *J. Cell Biol.* 122: 961-972 (1993); Sap *et al.*, *Mol. Cell. Biol.* 14: 1-9 (1994)). Interestingly, PTP κ and PTP μ do not interact with each other, despite their structural similarity (Zondag *et al.*, *J. Biol. Chem.* 270: 14247-14250 (1995)). From the studies described above it is apparent that PTPases may play an important role in regulating normal cell growth. However, as pointed out above, recent studies indicate that PTPases may also function as positive mediators of intracellular signalling and thereby induce or enhance mitogenic responses. Increased activity of certain PTPases might therefore result in cellular transformation and tumor formation. Indeed, in one study over-expression of PTP α was found to lead to transformation of rat embryo fibroblasts (Zheng, *supra*). In addition, a novel PTP, SAP-1, was found to be highly expressed in pancreatic and colorectal cancer cells. SAP-1 is mapped to chromosome 19 region q13.4 and might be related to carcinoembryonic antigen mapped to 19q13.2 (Uchida *et al.*, *J. Biol. Chem.* 269: 12220-12228 (1994)). Further, the dsPTPase, cdc25, dephosphorylates cdc2 at

Thr14/Tyr-15 and thereby functions as positive regulator of mitosis (reviewed by Hunter, *Cell* 80: 225-236 (1995)). Inhibitors of specific PTPases are therefore likely to be of significant therapeutic value in the treatment of certain forms of cancer.

5 PTPases: platelet aggregation

Recent studies indicate that PTPases are centrally involved in platelet aggregation. Agonist-induced platelet activation results in calpain-catalyzed cleavage of PTP1B with a concomitant 2-fold stimulation of PTPase activity (Frangioni *et al.*, *EMBO J.* 12: 4843-4856 (1993)). The
10 cleavage of PTP1B leads to subcellular relocation of the enzyme and correlates with the transition from reversible to irreversible platelet aggregation in platelet-rich plasma. In addition, the SH2 domain containing PTPase, PTP1C/SH-PTP1, was found to translocate to the cytoskeleton in platelets after thrombin stimulation in an aggregation-dependent manner (Li *et al.*, *FEBS Lett.* 343: 89-93 (1994)).

15 Although some details in the above two studies were recently questioned there is over-all agreement that PTP1B and PTP1C play significant functional roles in platelet aggregation (Ezumi *et al.*, *J. Biol. Chem.* 270: 11927-11934 (1995)). In accordance with these observations, treatment of platelets with the PTPase inhibitor pervanadate leads to
20 significant increase in tyrosine phosphorylation, secretion and aggregation (Pumiglia *et al.*, *Biochem. J.* 286: 441-449 (1992)).

PTPases: osteoporosis

25 The rate of bone formation is determined by the number and the activity of osteoblasts, which in turn are determined by the rate of proliferation and differentiation of osteoblast progenitor cells, respectively. Histomorphometric studies indicate that the osteoblast number is the primary determinant of the rate of bone formation in humans (Gruber *et al.*, *Mineral Electrolyte Metab.* 12: 246-254 (1987); reviewed in Lau *et al.*, *Biochem. J.* 257: 23-36
30 (1989)). Acid phosphatases/PTPases may be involved in negative regulation of osteoblast proliferation. Thus, fluoride, which has phosphatase inhibitory activity, has been found to increase spinal bone density in osteoporotics by increasing osteoblast proliferation (Lau *et*

al., supra). Consistent with this observation, an osteoblastic acid phosphatase with PTPase activity was found to be highly sensitive to mitogenic concentrations of fluoride (Lau *et al.*, *J. Biol. Chem.* 260: 4653-4660 (1985); Lau *et al.*, *J. Biol. Chem.* 262: 1389-1397 (1987); Lau *et al.*, *Adv. Protein Phosphatases* 4: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when the osteoblast-like cell line UMR 106.06 was grown on collagen type-I matrix compared to uncoated tissue culture plates. Since a significant increase in PTPase activity was observed in density-dependent growth arrested fibroblasts (Pallen and Tong, *Proc. Natl. Acad. Sci.* 88: 6996-7000 (1991)), it might be speculated that the increased PTPase activity directly inhibits cell growth. The mitogenic action of fluoride and other phosphatase inhibitors (molybdate and vanadate) may thus be explained by their inhibition of acid phosphatases/PTPases that negatively regulate the cell proliferation of osteoblasts. The complex nature of the involvement of PTPases in bone formation is further suggested by the recent identification of a novel parathyroid regulated, receptor-like PTPase, OST-PTP, expressed in bone and testis (Mauro *et al.*, *J. Biol. Chem.* 269: 30659-30667 (1994)). OST-PTP is up-regulated following differentiation and matrix formation of primary osteoblasts and subsequently down-regulated in the osteoblasts which are actively mineralizing bone in culture. It may be hypothesized that PTPase inhibitors may prevent differentiation via inhibition of OST-PTP or other PTPases thereby leading to continued proliferation. This would be in agreement with the above-mentioned effects of fluoride and the observation that the tyrosine phosphatase inhibitor orthovanadate appears to enhance osteoblast proliferation and matrix formation (Lau *et al.*, *Endocrinology* 116: 2463-2468 (1988)). In addition, it was recently observed that vanadate, vanadyl and pervanadate all increased the growth of the osteoblast-like cell line UMR106. Vanadyl and pervanadate were stronger stimulators of cell growth than vanadate. Only vanadate was able to regulate the cell differentiation as measured by cell alkaline phosphatase activity (Cortizo *et al.*, *Mol. Cell. Biochem.* 145: 97-102 (1995)).

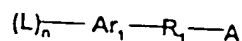
PTPases: microorganisms

Dixon and coworkers have called attention to the fact that PTPases may be a key element in the pathogenic properties of *Yersinia* (reviewed in Clemens *et al.* *Molecular Microbiology* 5: 2617-2620 (1991)). This finding was rather surprising since tyrosine phosphatase is thought to

be absent in bacteria. The genus *Yersinia* comprises 3 species: *Y. pestis* (responsible for the bubonic plague), *Y. pseudotuberculosis* and *Y. enterocolitica* (causing enteritis and mesenteric lymphadenitis). Interestingly, a dual-specificity phosphatase, VH1, has been identified in Vaccinia virus (Guan et al., *Nature* 350: 359-263 (1991)). These observations
 5 indicate that PTPases may play critical roles in microbial and parasitic infections, and they further point to PTPase inhibitors as a novel, putative treatment principle of infectious diseases.

SUMMARY OF THE INVENTION

10 The inventors have identified a novel class of compounds that has the capacity to modulate the activity of molecules with tyrosine recognition units, including PTPases, preferably a selective modulation. In one aspect, the present invention relates to novel organic compounds thereof of general formula (I)



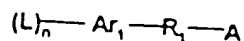
(I)

wherein

(L)_n, n, Ar₁, R₁ and A are defined as below

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel organic compounds thereof of formula (I)



(I)

wherein

25 n is 1, 2, 3, 4, or 5 and (L)_n represents up to five (5) substituents which independently of each other are hydrogen, C₁₋₆-alkyl, C₁₋₆-alkoxy, C₁₋₆-alkylthio, hydroxy, halogen, trihalogenomethyl, hydroxy-C₁₋₆-alkyl, amino-C₁₋₆-alkyl, -COR₂, -NO₂, -CN, -CHO,
 30

CO₂R₁

-C₁₋₆alkanoyloxy, carbamoyl, -NR₅R₆, aryloxy optionally substituted;

R₂ is C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted, -OH, -NR₃R₄ wherein R₃ and R₄ independently of each other are hydrogen, C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted;

5

R₅ and R₆ are independently of each other hydrogen or C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted or -COZ₁ wherein Z₁ is C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted;

10 or L is A₁-Y₁-(W₁)-X-(W₂)-Y₂ wherein X is a chemical bond, -CO, -CONR₇, -NR₇CO, -NR₇, -O-, -S-, -SO, or -SO₂;

Y₁ and Y₂ are independently a chemical bond, -O-, -S-, or -NR₇;

R₇ is hydrogen, C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -COZ₂ wherein Z₂ is C₁₋₆alkyl, aryl optionally substituted, aralkyl

15 optionally substituted;

W₁ and W₂ are independently a chemical bond or saturated or unsaturated C₁₋₆alkylene;

20 A₁ is aryl optionally substituted, heteroaryl optionally substituted, biaryl optionally substituted, arylheteroaryl optionally substituted, -NR₈R₉ wherein R₈ and R₉ independently are hydrogen, C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -COZ₃ wherein Z₃ is C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted

or

25 when R₈ and R₉ together with the nitrogen atom forms a ring system A₁ is a saturated or partially saturated heterocyclic ring system optionally substituted with

C₁₋₆alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -OH, C₁₋₆alkoxy, C₁₋₆alkylthio, hydroxy-C₁₋₆alkyl, amino-C₁₋₆alkyl, -COZ₄ wherein Z₄ is -OH, C₁₋₆alkyl, -NR₁₀R₁₁ wherein R₁₀ and R₁₁ independently are hydrogen, C₁₋

30 6-alkyl;

any benzene substituted

Aralk any substitute on benzene

R_1 is a linker selected from a chemical bond, $-C_{1-6}\text{-alkyl-}$, $-O(CH_2)_m-$, $-NR_{12}-$, $-\text{CONR}_{12}-$, $-NR_{13}\text{CO-}$, $-\text{SO}_2\text{NR}_{14}-$, $-\text{NR}_{15}\text{SO}_2-$, $-\text{CR}_{16}=\text{CR}_{17}-$, $-\text{CH=}$, $-\text{CHR}_{17}$, $-\text{CH}_2-$, $-\text{CHF-}$, $-\text{CF}_2-$, $-\text{SO}_2-$; R_{12} , R_{13} , R_{14} , R_{15} , R_{16} and R_{17} are hydrogen, $C_{1-6}\text{-alkyl}$, aralkyl and m is 1, 2, or 3;

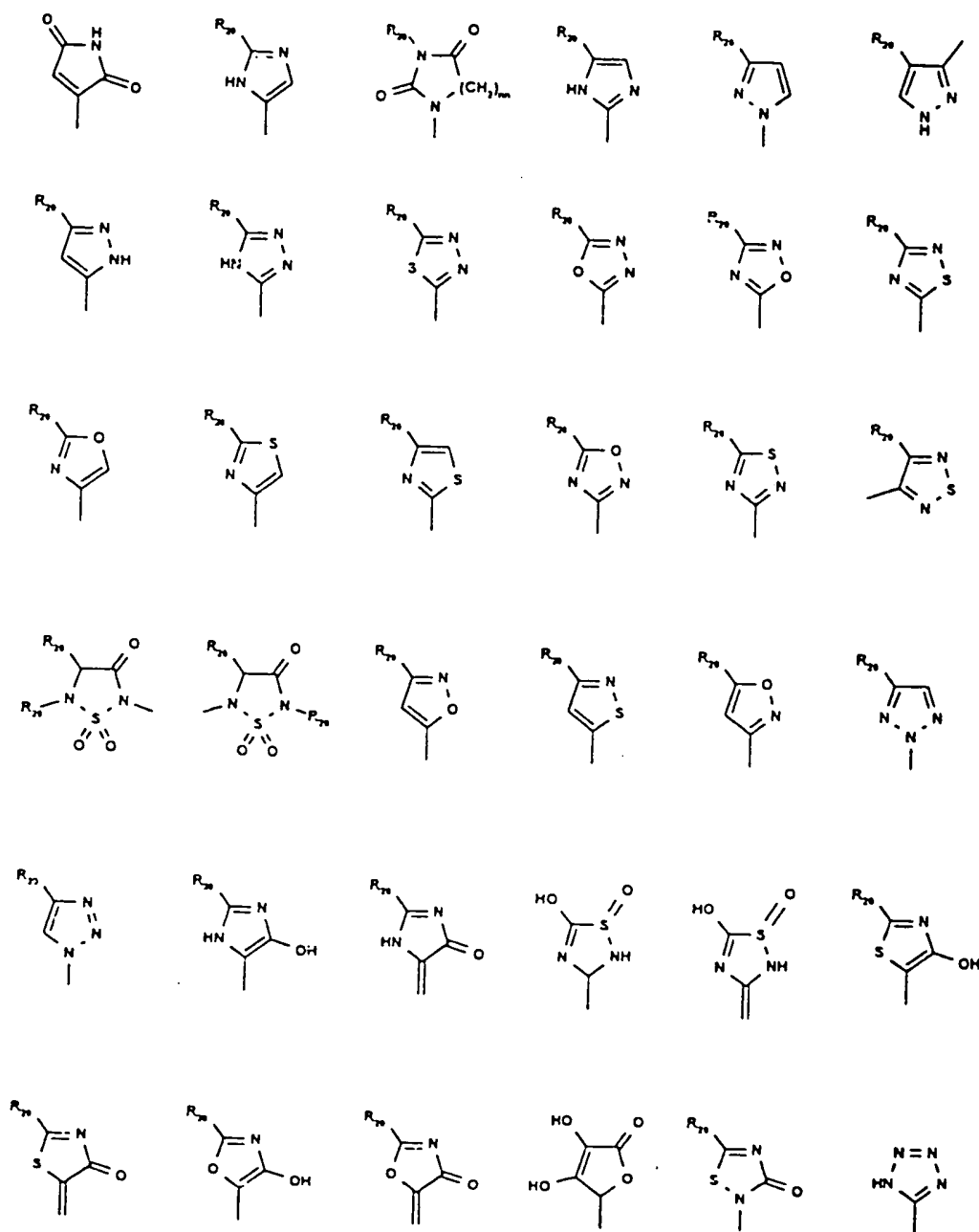
5

A is $-\text{PO}(\text{OR}_{18})(\text{OR}_{19})$, $-\text{NH-SO}_3\text{H}$, $-\text{NH-SO}_2\text{-CH}_3$, $-\text{NH-SO}_2\text{-CF}_3$, $-\text{CO-NH-OH}$ or a heterocycle as shown in scheme 1 wherein the point of attachment is indicated with a | (single bond) or || (double bond)

10

15

Scheme 1



optionally substituted by hydrogen, halogen, C_{1-6} -alkyl optionally substituted by phenyl
optionally substituted by C_{1-6} -alkoxy, C_{1-6} -alkylthio; $-COOX^1$ wherein X^1 is
5 C_{1-6} -alkyl optionally substituted by phenyl or benzyl optionally substituted;

R_{18} , and R_{19} independently are hydrogen, C_{1-6} -alkyl, phenyl, benzyl;

R_{20} is hydrogen, -OH, C_{1-6} -alkoxy, -SH, C_{1-6} -alkylthio, C_{1-6} -alkylcarbonyloxy, -COR₂₁, -SOR₂₂, -SO₂R₂₃, -NR₂₄R₂₅, -NHCN, halogen, trihalogenomethyl;

R_{21} , R_{22} , and R_{23} are -OR₂₆, C_{1-6} -alkyl, -NR₂₄R₂₅, trihalogenomethyl;

5 R_{24} and R_{25} independently are hydrogen, C_{1-6} -alkyl, -SO₂R₂₆, -COZ₅ wherein Z₅ is C_{1-6} -alkyl, trihalogenomethyl

R_{26} is hydrogen, C_{1-6} -alkyl, trihalogenomethyl;

nn is 1 or 2;

and Ar₁ is aryl or heteroaryl;

10

or a pharmaceutically acceptable salt thereof.

In the above-mentioned formula (I) aryl, heteroaryl, Ar₁ or A₁ are exemplified by the following examples. Specific examples of the aryl and biaryl residues include phenyl, biphenyl, indenyl,

15 fluorenyl, naphthyl (1-naphthyl, 2-naphthyl), anthracenyl (1-anthracenyl, 2-anthracenyl, 9-anthracenyl). Specific examples of the heteroaryl include pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl, 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), isoxazolyl (3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl), thiazolyl (2-thiazolyl,

20 4-thiazolyl, 5-thiazolyl), thiophenyl (2-thiophenyl, 3-thiophenyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl

25 (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl),

7-(2,3-dihydro-benzo[b]furanyl), benzo[b]thiophenyl (2-benzo[b]thiophenyl,

30 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl,

6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl),

5-(2,3-dihydro-benzo[b]thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazolyl (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl), piperidinyl (2-piperidinyl, 3-piperidinyl, 4-piperidinyl), pyrrolidinyl (1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl), morpholinyl (1-morpholinyl, 2-morpholinyl), piperazinyl (1-piperazinyl).

Specific examples of the arylheteroaryl residue include phenylpyridyl (2-phenylpyridyl, 3-phenylpyridyl, 4-phenylpyridyl), phenylpyrimidinyl (2-phenylpyrimidinyl, 4-phenylpyrimidinyl, 5-phenylpyrimidinyl, 6-phenylpyrimidinyl), phenylpyrazinyl, phenylpyridazinyl (3-phenylpyridazinyl, 4-phenylpyridazinyl, 5-phenylpyridazinyl).

The C₁₋₆ alkyl residues include aliphatic hydrocarbon residues, unsaturated aliphatic hydrocarbon residues, alicyclic hydrocarbon residues. Examples of the aliphatic hydrocarbon residues include saturated aliphatic hydrocarbon residues having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.butyl, tert.butyl, n-pentyl, isopentyl, neopentyl, tert.pentyl, n-hexyl, isohexyl. Example of the unsaturated aliphatic hydrocarbon residues include those having 2 to 6 carbon atoms such as ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 2-methyl-1-propenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 3-methyl-2-butenyl, 1-hexenyl, 3-hexenyl, 2,4-hexadienyl, 5-hexenyl, ethynyl, 1-propionyl, 2-propionyl, 1-butyryl, 2-butyryl, 3-butyryl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl.

1-hexynyl, 3-hexynyl, 2,4-hexadiynyl, 5-hexynyl. Examples of the alicyclic hydrocarbon residue include saturated alicyclic hydrocarbon residues having 3 to 6 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl; and C₅₋₆ unsaturated alicyclic hydrocarbon residues having 5 to 6 carbon atoms such as 1-cyclopentenyl, 2-cyclopentenyl, 3-cyclopentenyl, 1-cyclohexenyl, 2-cyclohexenyl, 3-cyclohexenyl.

The C₁₋₆-alkoxy residues include aliphatic hydrocarbon residues connected to an oxygen atom. Examples of the aliphatic hydrocarbon residues include saturated aliphatic hydrocarbon residues having 1 to 6 carbon atoms such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec.butoxy, tert.butoxy, pentoxy, isopentoxy, neopentoxy, tert.pentoxy, hexyloxy, isohexyloxy.

The C₁₋₆-alkylthio residues include aliphatic hydrocarbon residues connected to a sulphur atom. Examples of the aliphatic hydrocarbon residues include saturated aliphatic hydrocarbon residues having 1 to 6 carbon atoms such as methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, sec.butylthio, tert.butylthio, pentylthio, isopentylthio, neopentylthio, tert.pentylthio, hexylthio, isohexylthio.

The C₁₋₆-alkoxycarbonyl residues include a C₁₋₆-alkoxy residue connected to a carbonyl residue such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, and tert-butoxycarbonyl.

The C₁₋₆-alkylcarbonyloxy residues include a C₁₋₆-alkyl residue connected to a carbonyloxy residue such as acetic acid, propionic acid, butyric acid.

The C₁₋₆-alkanoyloxy residues include an acyl residue connected to an oxygen atom wherein the acyl residue is an aliphatic hydrocarbon residues connected to a carbonyl residue such as acetyloxy, propionyloxy, isopropionyloxy.

The aralkyl residue include an aryl residue connected to a C₁₋₆-alkyl residue e.g. phenyl alkyls having 7 to 9 carbon atoms such as benzyl, phenethyl, 1-phenylethyl, 3-phenylpropyl,

2-phenylpropyl and 1-phenylpropyl; and naphthyl alkyl having 11 to 13 carbon atoms such as 1-naphthylmethyl, 1-naphthylethyl, 2-naphthylmethyl, and 2-naphthylethyl.

Aryloxy include an aryl connected to an oxygen atom such as phenyloxy, naphthyloxy.

Aralkyloxy include an aralkyl connected to an oxygen atom such as benzyloxy, phenethyloxy, naphthylmethyloxy.

Biaryl include an aryl connected to an aryl residue such as biphenyl, 1-phenylnaphthyl, 2-phenylnaphthyl.

Biaryloxy include an biaryl connected to an oxygen atom such as biphenyloxy, 4-(naphthalene-1-yl)phenoxy, 4-(naphthalene-2-yl)phenoxy.

The heteroaryl residue is a 5- or 6-membered aromatic ring, which can be fused to one or more phenyl rings and contains, besides carbon atoms, 1 to 4 atoms selected from N, O, and S as atoms constituting the ring, which is bonded through carbon atoms such as defined above.

The halogen residue include fluorine, chlorine, bromine, and iodine.

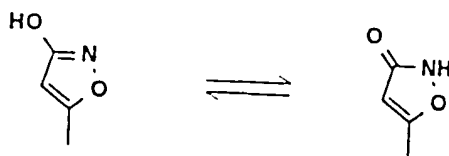
The term "optionally substituted" means an aryl residue, a heteroaryl residue, or a C₁₋₆ alkyl residue that may be unsubstituted or may have 1 or more preferably 1 to 5 substituents, which are the same as or different from one another. Examples of these substituents include, halogen (fluorine, chlorine, bromine, iodine), hydroxyl, cyano, nitro, trifluoromethyl, carbamoyl, C₁₋₄-acyl (e.g. acetyl, propionyl, isopropionyl), C₁₋₆-alkoxy (e.g. methoxy, ethoxy, propoxy, isopropoxy, butoxy, and tert.butoxy), C₁₋₆-alkyl (e.g. methyl, ethyl, propyl, cyclopropyl, isopropyl, butyl, and tert.butyl), C₁₋₆-alkoxycarbonyl (e.g. ones having 2 to 6 carbon atoms such as methoxycarbonyl, ethoxycarbonyl, and propoxycarbonyl), C₁₋₆-alkanoyloxy (e.g. ones having 2 to 6 carbon atoms such as acetyloxy, propionyloxy, isopropionyloxy), C₁₋₄-alkylthio (e.g. ones having 1 to 4 carbon atoms such as methylthio,

ethylthio, propylthio, and isopropylthio), C₁₋₄-alkylamino (e.g. one having 1 to 4 carbon atoms such as methylamino, ethylamino, dimethylamino, and 1-pyrrolidinyl), heteroaryl (as exemplified above), aryloxy (e.g. phenyloxy), and an aralkyloxy (e.g. benzyloxy).

- 5 The compounds of formula (I) may exist as geometric and optical isomers and all isomers and mixtures thereof are included herein. Isomers may be separated by means of standard methods such as chromatographic techniques or fractionated crystallisation of e.g. suitable salts.

It is to be understood that the heterocyclic moieties depicted throughout this application is

- 10 capable of undergoing tautomerisation. Example of tautomerisation is given by the following example, thus:



- 15 The compounds according to the invention may optionally exist as pharmaceutically acceptable salts comprising acid addition salts or metal salts or - optionally alkylated - ammonium salts.

- Examples of such salts include the alkali metal or amine salts of 1H- or 2H-tetrazoles of this invention, such as the sodium, potassium, C₁₋₆-alkylamine, di (C₁₋₆-alkyl) amine, tri (C₁₋₆-alkyl) amine and the four (4) corresponding omega-hydroxy analogues (e.g. methylamine, ethylamine, propylamine, dimethylamine, diethylamine, dipropylamine, trimethylamine, triethylamine, tripropylamine, di(hydroxyethyl)amine, and the like;
- 20 inorganic and organic acid addition salts such as hydrochloride, hydrobromide, sulphate, phosphate, acetate, fumarate, maleate, citrate, lactate, tartrate, oxalate or similar
- 25 pharmaceutically acceptable inorganic or organic acid addition salts, and include the pharmaceutically acceptable salts listed in *Journal of Pharmaceutical Science* 66: 2 (1977) which are hereby incorporated by reference.

The compounds of formula (I) may be prepared by art-recognised procedures from known compounds or readily preparable intermediates. An exemplary general procedure is as follows:

5 Method A:

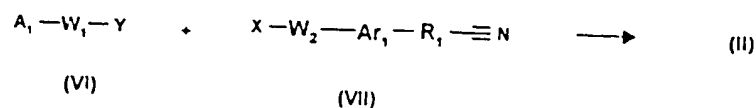
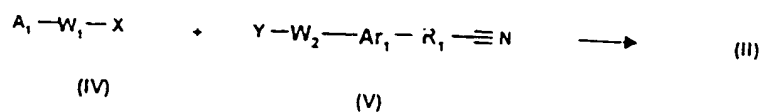


By allowing a compound of formula (II) wherein $(L)_n$, Ar_1 , R_1 , and n are as defined above to
 10 react with an azide of formula (III) wherein M is an alkali metal (*J. Am. Chem. Soc.* 80: 3908 (1958)), trialkyltin $Sn(R_{18})_3$ wherein R_{18} is C_{1-4} -alkyl (*J. Org. Chem.* 56: 2395 (1991)), or trialkylsilyl $Si(R_{19})_3$ wherein R_{19} is C_{1-4} -alkyl (*Tetrahedron Lett.* 34: 8011 (1993)), a tetrazole derivative of formula (I) wherein A is a tetrazol is produced. These cyclisation reactions may
 15 be carried out in a solvent such as dimethylformamide (DMF), tetrahydrofuran (THF) or toluene at temperatures ranging from 80 °C to 150 °C for 1 to 60 hours.

The tetrazole derivatives and their salts thus obtained can be isolated and purified by known means of separation and purification such as concentration, concentration under reduced pressure, crystallisation, recrystallisation, extraction and chromatography.
 20

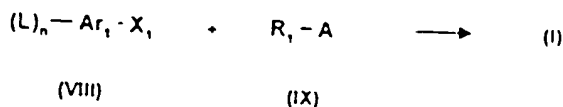
The nitrile derivatives (II) used as starting materials in the method A of this invention can be produced by, for example, the following manner.

26



- By allowing a compound of formula (IV) wherein A_1 and W_1 are as defined above, X is OH , SH , and NHR_7 , wherein R_7 is as defined above to react with a compound of formula (V) wherein W_2 and Ar_1 are as defined above and Y is a suitable leaving group such as halogen, *p*-toluene sulphonate, mesylate or hydroxy; or by allowing a compound of formula (VI) wherein A_1 and W_1 are as defined above and Y is a suitable leaving group such as halogen, *p*-toluene sulphonate, mesylate or hydroxy to react with a compound of formula (VII) wherein W_2 and Ar_1 are as defined above, X is OH , SH , and NHR_7 , wherein R_7 is as defined above.
- These reactions may be carried out in a solvent such as *N*-methyl pyrrolidone (NMP), dimethylformamide (DMF), tetrahydrofuran (THF), acetone, dibutyl ether, 2-butanone, methyl *tert*-butyl ether, methyl ethyl ketone, ethyl acetate or toluene in the presence of a base e.g. potassium carbonate or sodium hydride and a catalyst, e.g. an alkali metal iodide, copper or a copper salt e.g. (CuCl , CuBr , CuI , or Cu_2O) or in the case of a Mitsunobu reaction (for a review see, O. Mitsunobu, *Synthesis*, 1 (1981)) in the presence of e.g. diethyl azodicarboxylate and triphenylphosphine at temperatures ranging from -10°C to 200°C for 1 to 60 hours.

Method B:

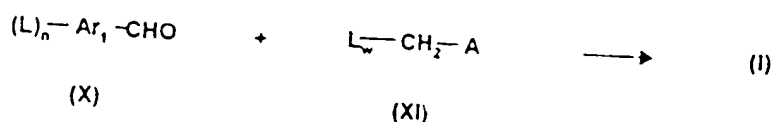


By allowing a compound of formula (VIII), wherein $(L)_n$, n , and Ar_1 are as defined above and X_1 is a suitable leaving group such as bromo, iodo or trifluoromethane sulfonyloxy to react with a compound of formula (IX) wherein R_1 is $CH_2=CH$ and A are as defined above.

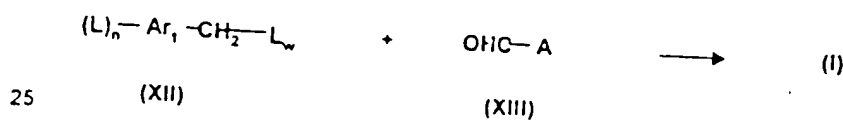
- 5 These reactions may be carried out in a solvent such as triethylamine (TEA), methanol, ethanol or dimethylsulfoxid (DMSO) in the presence of a palladium catalyst, e.g. (Pd/C, Pd/Al₂O₃, Pd/BaSO₄, Pd/SiO₂ or Pd(OAc)₂ (a Heck reaction)) and a triaryl-phosphine catalyst as e.g. (triphenyl-phosphine or tri-*o*-tolyl-phosphine) at temperatures ranging from 50 °C to 150 °C for 1 to 60 hours.

10

Method C:



- 15 By allowing a compound of formula (X), wherein $(L)_n$, n , and Ar_1 are as defined above to react with a compound of formula (XI) wherein A is as defined above and L_w is trimethylsilyl (a Peterson reaction), triphenyl-phosphonium (a Wittig reaction), diethyl phosphate (a modified Wittig reaction) or carbonyloxyC₁₋₆-alkyl (e.g. -COOEt or -COOMe) or,
- 20 by allowing a compound of formula (XII), wherein $(L)_n$, n , and Ar_1 are as defined above and L_w is trimethylsilyl (a Peterson reaction), triphenyl-phosphonium (a Wittig reaction), diethyl phosphate (a modified Wittig reaction) or carbonyloxyC₁₋₆-alkyl (e.g. -COOEt or -COOMe) to react with a compound of formula (XIII) wherein A is as defined above.

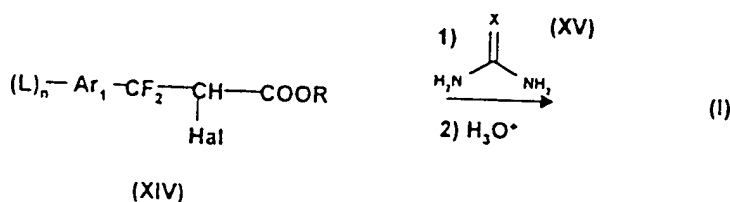


25

These reactions may be carried out in a solvent such as methanol, ethanol, tetrahydrofuran (THF), toluene, N,N-dimethylformamide (DMF) or dimethylsulfoxid (DMSO) in the presence of a base such as triethylamine, pyridine, piperidine, sodium hydride, sodium methoxide,

sodium ethoxide, potassium tert-butoxide, lithium diisopropylamide at temperatures ranging from -50 °C to 150 °C for 1 to 60 hours.

Method D:



By allowing a compound of formula (XIV), wherein (L)_n, n and Ar₁ are as defined above and Hal is chloro or bromo and R is C₁₋₆-alkyl to react with a compound of formula (XV) wherein X is O or S whereby a compound of formula (I) is produced wherein R₁ is CF₂ and A are 2,4-dihydroxy-oxazolidin-5-yl or 2,4-dihydroxy-thiazolidin-5-yl;

Compounds of formula (II), (IV) to (XV) may be prepared by methods familiar to those skilled in the art.

Under certain circumstances it may be necessary to protect the intermediates used in the above methods. The tetrazole group can, for example, be protected by a trityl group. Introduction and removal of such groups is e.g. described in "Protective Groups in Organic Synthesis" T.W. Greene and P.G.M. Wuts, ed. Second edition (1991).

In preferred embodiments, the compounds of the invention modulate the activity of protein tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s).

In one preferred embodiment the compounds of the invention act as inhibitors of PTPases, e.g. protein tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via interaction with regulatory PTPases, e.g. the signalling pathways of the insulin receptor, the IGF-I receptor and other members of the insulin receptor family, the EGF-receptor family, the platelet-derived growth factor receptor family, the nerve growth factor

receptor family, the hepatocyte growth factor receptor family, the growth hormone receptor family and members of other receptor-type tyrosine kinase families. Further preferred embodiments of the inventions is modulation of non-receptor tyrosine kinase signalling through modulation of regulatory PTPases, e.g. modulation of members of the Src kinase family. One type of preferred embodiments of the inventions relate to modulation of the activity of PTPases that negatively regulate signal transduction pathways. Another type of preferred embodiments of the inventions relate to modulation of the activity of PTPases that positively regulate signal transduction pathways.

10 In a preferred embodiment the compounds of the invention act as modulators of the active site of PTPases. In another preferred embodiment the compounds of the invention modulate the activity of PTPases via interaction with structures positioned outside of the active sites of the enzymes, preferably SH2 domains. Further preferred embodiments include modulation of signal transduction pathways via binding of the compounds of the invention to SH2 domains or PTB domains of non-PTPase signalling molecules.

Other preferred embodiments include use of the compounds of the invention for modulation of cell-cell interactions as well as cell-matrix interactions.

20 As a preferred embodiment, the present invention include within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of formula (I) in association with a pharmaceutical carrier or diluent. Optionally, the pharmaceutical composition can comprise at least one of the compounds of formula (I) combined with compounds exhibiting a different activity, e.g. an antibiotic or other pharmacologically active material.

30 As a preferred embodiment, the compounds of the invention may be used as therapeutics to inhibit of PTPases involved in regulation of the insulin receptor tyrosine kinase signalling pathway in patients with type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, and obesity. Further preferred embodiments include use of the compounds of the invention for treatment of disorders with general or specific dysfunction's of PTPase activity,

e.g. proliferative disorders such as psoriasis and neoplastic diseases. As another embodiment, the compounds of the invention may be used in pharmaceutical preparations for treatment of osteoporosis.

- 5 Preferred embodiments of the invention further include use of compound of formula (I) in pharmaceutical preparations to increase the secretion or action of growth hormone and its analogous or somatomedins including IGF-1 and IGF-2 by modulating the activity of PTPases or other signal transduction molecules with affinity for phosphotyrosine involved controlling or inducing the action of these hormones or any regulating molecule.

10

- To those skilled in the art, it is well known that the current and potential uses of growth hormone in humans are varied and multi-tudinous. Thus, compounds of the invention can be administered for purposes of stimulating the release of growth hormone from the pituitary or increase its action on target tissues thereby leading to similar effects or uses as growth
- 15 hormone itself. The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of catabolic side effects of glucocorticoids; treatment of osteoporosis, stimulation of the immune system; treatment of retardation, acceleration of wound healing; accelerating bone fracture repair; treatment of growth retardation; treating renal failure or insufficiency resulting in growth retardation; treatment of
- 20 physiological short stature including growth hormone deficient children and short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treating growth retardation associated with the Prader-Willi syndrome and Turner's syndrome; accelerating the recovery and reducing hospitalization of burn patients; treatment of intrauterine growth retardation, skeletal dysplasia, hypercortisolism and Cushings
- 25 syndrome; induction of pulsatile growth hormone release; replacement of growth hormone in stressed patients; treatment of osteochondro-dysplasias, Noonans syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation; treatment of pulmonary dysfunction and ventilator dependency; attenuation of protein catabolic responses after major surgery; reducing cachexia and protein loss due to chronic
- 30 illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidio-blastosis; Adjuvant treatment for ovulation induction; stimulation of thymic development and prevention the age-related decline of thymic function; treatment of immunosuppressed patients;

improvement in muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal hemeostasis in the frail elderly; stimulation of osteoblasts, bone remodelling and cartilage growth; stimulation of the immune system in companion animals and treatment of disorder of aging in companion animals; growth promotant in livestock and
5 stimulation of wool growth in sheep.

The compounds of the invention may be used in pharmaceutical preparations for treatment of various disorders of the immune system, either as a stimulant or suppressor of normal or perturbed immune functions, including autoimmune reactions. Further embodiments of the
10 invention include use of the compounds of the invention for treatment of allergic reactions, e.g. asthma, dermal reactions, conjunctivitis.

In another embodiment compounds of the invention may be used in pharmaceutical preparations for prevention or induction of platelet aggregation.
15

In yet another embodiment, compounds of the invention may be used in pharmaceutical preparations for treatment of infectious disorders. In particular, the compounds of the invention may be used for treatment of infectious disorders caused by *Yersinia* and other bacteria as well as disorders caused by viruses or other micro-organisms.
20

Compounds of the invention may additionally be used for treatment or prevention of diseases in animals, including commercially important animals.

Also included in the present invention is a process for isolation of PTPases via affinity
25 purification procedures based on the use of immobilised compounds of the invention using procedures well-known to those skilled in the art.

The invention is further directed to a method for detecting the presence of PTPases in cell or in a subject comprising:

- (a) contacting said cell or an extract thereof with labelled compounds of the invention.
- 30 (b) detecting the binding of the compounds of the invention or measuring the quantity bound, thereby detecting the presence or measuring the quantity of certain PTPases.

The invention further relates to analysis and identification of the specific functions of certain PTPases by modulating their activity by using compounds of the invention in cellular assay systems or in whole animals.

5 DEFINITIONS

Signal transduction is a collective term used to define all cellular processes that follow the activation of a given cell or tissue. Examples of signal transduction, which are not intended to be in any way limiting to the scope of the invention claimed, are cellular events that are
10 induced by polypeptide hormones and growth factors (e.g. insulin, insulin-like growth factors I and II, growth hormone, epidermal growth factor, platelet-derived growth factor), cytokines (e.g. inter-leukins), extracellular matrix components, and cell-cell interactions.

Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units.
15 are defined as areas or domains of proteins or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not intended to be in any way limiting to the scope of the invention claimed, are: PTPases, SH2 domains and PTB domains.

20 PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing proteins or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the invention claimed, are: 'classical' PTPases (intracellular PTPases (e.g. PTP1B, TC-PTP, PTP1C, PTP1D, PTPD1, PTPD2) and receptor-type PTPases (e.g. PTP α , PTP ϵ , PTP β , PTP γ , CD45, PTP κ , PTP μ), dual specificity phosphatases (VH1, VHR,
25 cdc25), LMW-PTPases or acid phosphatases.

Modulation of cellular processes is defined as the capacity of compounds of the invention to
1) either increase or decrease ongoing, normal or abnormal, signal transduction, 2) initiate normal signal transduction, and 3) initiate abnormal signal transduction.

30 Modulation of pTyr-mediated signal transduction/modulation of the activity of molecules with pTyr recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of proteins or glycoproteins with pTyr recognition units (e.g.

- PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a protein or glyco-protein with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples of modulation of pTyr-mediated signal transduction/modulation of the activity of molecules with
- 5 pTyr recognition units, which are not intended to be in any way limiting to the scope of the invention claimed, are: a) inhibition of PTPase activity leading to either increased or decreased signal transduction of ongoing cellular processes; b) inhibition of PTPase activity leading to initiation of normal or abnormal cellular activity; c) stimulation of PTPase activity leading to either increased or decreased signal transduction of ongoing cellular processes; d)
- 10 stimulation of PTPase activity leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to proteins or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to proteins or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

15

A subject is defined as any mammalian species, including humans.

Pharmacological Compositions

- 20 For the above indications the dosage will vary depending on the compound of formula (I) employed, on the mode of administration and on the therapy desired. However, in general, satisfactory results are obtained with a dosage of from about 0.5 mg to about 1000 mg, preferably from about 1 mg to about 500 mg of compounds of formula (I), conveniently given from 1 to 5 times daily, optionally in sustained release form. Usually, dosage forms suitable
- 25 for oral administration comprise from about 0.5 mg to about 1000 mg, preferably from about 1 mg to about 500 mg of the compounds of formula (I) admixed with a pharmaceutical carrier or diluent.

- The compounds of formula (I) may be administered in a pharmaceutically acceptable acid
- 30 addition salt form or where possible as a metal or a C₁₋₆-alkylammonium salt. Such salt forms exhibit approximately the same order of activity as the free acid forms.

This invention also relates to pharmaceutical compositions comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof and, usually, such compositions also contain a pharmaceutical carrier or diluent. The compositions containing the compounds of this invention may be prepared by conventional techniques and appear in conventional
5 forms, for example capsules, tablets, solutions or suspensions.

The pharmaceutical carrier employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, terra alba, sucrose, talc, gelatine, agar, pectin, acacia, magnesium stearate and stearic acid. Examples of liquid carriers are syrup, peanut oil, olive
10 oil and water.

Similarly, the carrier or diluent may include any time delay material known to the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

15 If a solid carrier for oral administration is used, the preparation can be tableted, placed in a hard gelatine capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or
20 solution.

Generally, the compounds of this invention are dispensed in unit dosage form comprising 10-200 mg of active ingredient in or together with a pharmaceutically acceptable carrier per unit dosage.
25

The dosage of the compounds according to this invention is 1-500 mg/day, e.g. about 100 mg per dose, when administered to patients, e.g. humans, as a drug.

A typical tablet which may be prepared by conventional tableting techniques contains
30

35

Core:

Active compound (as free compound 100 mg
or salt thereof)

	Colloidal silicon dioxide (Areosil [®])	1.5 mg
5	Cellulose, microcryst. (Avicel [®])	70 mg
	Modified cellulose gum (Ac-Di-Sol [®])	7.5 mg
	Magnesium stearate	

Coating:

10	HPMC	approx.	9 mg
	Mywacett [®] 9-40 T	approx.	0.9 mg

Acylated monoglyceride used as plasticiser for film coating.

- 15 The route of administration may be any route which effectively transports the active compound to the appropriate or desired site of action, such as oral or parenteral e.g. rectal, transdermal, subcutaneous, intranasal, intramuscular, topical, intravenous, intraurethral, ophthalmic solution or an ointment, the oral route being preferred.

20

EXAMPLES

The process for preparing compounds of formula (I) and preparations containing them is further illustrated in the following examples, which, however, are not to be construed as

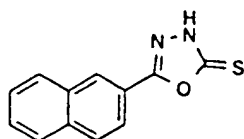
25 limiting.

- Hereinafter, TLC is thin layer chromatography, CDCl₃ is deuterio chloroform and DMSO-d₆ is hexadeuterio dimethylsulfoxid. The structures of the compounds are confirmed by either elemental analysis or NMR, where peaks assigned to characteristic protons in the title
- 30 compounds are presented where appropriate. ¹H NMR shifts (δ_H) are given in parts per million (ppm) down field from tetramethylsilane as internal reference standard. M.p. is melting point and is given in °C and is not corrected. Column chromatography was carried out using

the technique described by W.C. Still *et al.*, *J. Org. Chem.* 43: 2923 (1978) on Merck silica gel 60 (Art. 9385). HPLC analyses were performed using 5µm C18 4 x 250 mm column eluted with various mixtures of water and acetonitrile, flow = 1 ml/min, as described in the experimental section.

5

Compounds used as starting material are either known compounds or compounds which can readily be prepared by methods known per se.

10 EXAMPLE 115 5-Naphthalen-2-yl-3H-[1,3,4]oxadiazole-2-thione

To a solution of 2-naphthyl carboxylic acid ethyl ester (2.0 g, 9.99 mmol) in absolute ethanol (30 ml) was added hydrazine hydrate (4.85 ml, 99.9 mmol) and the reaction mixture was heated at reflux temperature for 72 h. The reaction mixture was cooled and the precipitate was filtered off, washed with 96 % ethanol (2 x 10 ml) and diethyl ether (3 x 10 ml), dried in vacuo at 50 °C which afforded 0.9 g (48%) of naphthalene-2-carboxylic acid hydrazide as a solid.

To a stirred solution of the above hydrazide (1.0 g, 5.37 mmol) in methanol (20 ml) was added potassium hydroxide (0.33 g, 5.88 mmol) and carbondisulfide (0.94 g, 12.35 mmol) at 0 °C. The reaction mixture was stirred at reflux temperature for 7 h, cooled and quenched with water (100 ml). The resultant mixture was washed with diethyl ether (50 ml) and acidified to pH = 1 with 1 N hydrochloric acid. The precipitate was filtered off, washed with water (2 x 20 ml) and heptane (2 x 20 ml) and dried in vacuo at 50 °C which afforded 0.77 g (63%) of the title compound as a solid.

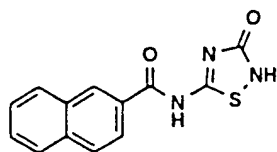
Calculated for $C_{12}H_8N_2OS$:

C, 63.14 %; H, 3.53 %; N, 12.27 %. Found

C, 62.86 %; H, 3.47 %; N, 12.17 %.

5

EXAMPLE 2



10

Naphthalene-2-carboxylic acid (3-oxo-2,3-dihydro-[1,2,4]thiadiazol-5-yl)-amide

To a mixture of 2-naphthyl carboxylic acid (5.0 g, 29.0 mmol) and 2 drops of N,N'-
15 dimethylformamide in dry tetrahydrofuran (50 ml) was added dropwise thionyl chloride
(6.3 ml, 87 mmol) and the resulting reaction mixture was stirred at reflux temperature for
3 h. The volatiles were evaporated in vacuo and the solid residue was redissolved in dry
tetrahydrofuran (30 ml) and added dropwise to a solution of potassium thiocyanate (2.9
g, 30 mmol) in acetone (40 ml). The reaction mixture was stirred at room temperature for
20 16 h. The reaction mixture was quenched with water (250 ml) and extracted with diethyl
ether (2 x 100 ml). The combined organic extracts were washed with saturated aqueous
sodium chloride (2 x 80 ml), dried ($MgSO_4$), filtered and evaporated in vacuo affording
5.2 g (84%) of naphthalene-2-carbonyl isothiocyanate.

25 To a solution of the above isothiocyanate (5.0 g, 23 mmol) in acetone (100 ml) was ad-
ded urea (1.44 g, 24 mmol) and the resulting mixture was heated at reflux temperature
for 4 h. An additional portion of urea (0.8 g, 13.3 mmol) was added and the reaction
mixture was heated at reflux temperature for 17 h. The cooled reaction mixture was qu-
enched by addition of water (150 ml) and stirred for 15 min. The precipitate was filtered

off and washed with water (2 x 25 ml), dried in vacuo at 50 °C affording 5.1 g (80%) of naphthalene-2-carboxylic acid ureidocarbothioyl-amide as a solid.

To a stirred solution of the above ureidocarbothioyl-amide (4.5 g, 0.017 mol) in ethanol (40 ml) at 35 °C was added dropwise a 1 N solution of bromine in dichloromethane (17 ml) during 10 min. The resulting reaction mixture was stirred for 0.5 h at room temperature. Water (50 ml) was added and the precipitate was filtered off, washed with water (2 x 50 ml) and diethyl ether (2 x 50 ml) and dried in vacuo at 50 °C affording 3.1 g (69%) of the title compound as a solid.

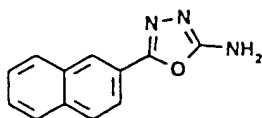
m.p.: > 250 °C.

Calculated for $C_{13}H_9N_3O_2S$:

C, 57.56 %; H, 3.34 %; N, 15.59 %. Found

C, 57.59 %; H, 3.34 %; N, 15.07 %.

EXAMPLE 3



5-Naphthalen-2-yl-[1,3,4]oxadiazol-2-ylamine

To a stirred solution of naphthalene-2-carboxylic acid hydrazide (0.6 g, 3.22 mmol, prepared as described in example 1) in dioxane (20 ml) was added a solution of sodium hydrogen carbonate (0.27 g, 3.22 mmol) in water (15 ml) and the resulting mixture was stirred for 5 min. To the reaction mixture was added cyanogen bromide (0.35 g, 3.3 mmol) and the mixture was stirred for 3 h at room temperature. The precipitate was filtered off and washed with diethyl ether (2 x 15 ml) and dried in vacuo at 50 °C affording 0.4 g of crude product which was suspended in absolute ethanol (15 ml) and stirred at reflux temperature for 0.5 h. The cooled suspension was filtered and the filter cake was dried in vacuo at 50 °C affording 130 mg (20%) of the title compound as a solid.

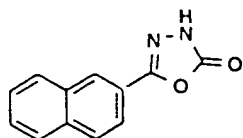
m.p.: 245-247 °C

Calculated for $C_{12}H_9N_3O$, $0.1 \times H_2O$:

5 C, 67.66 %; H, 4.35 %; N, 19.73 %. Found
C, 67.56 %; H, 4.21 %; N, 19.84 %.

EXAMPLE 4

10



5-Naphthalen-2-yl-3H-[1,3,4]oxadiazol-2-one

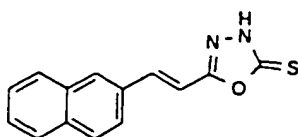
15

To a stirred mixture of naphthalene-2-carboxylic acid hydrazide (2.0 g, 10.7 mmol, prepared as described in example 1) and triethylamine (1.1 g, 10.7 mmol) in dry tetrahydrofuran (40 ml) was added carbonyl diimidazole (2.2 g, 13.4 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 17 h. The resulting
20 reaction mixture was evaporated in vacuo and to the residue was added water (50 ml) and ethyl acetate (50 ml). The phases were separated and the organic phase was washed with saturated aqueous sodium chloride (2 x 25 ml), dried ($MgSO_4$) and filtered and evaporated in vacuo affording 2.2 g of crude product which was recrystallised from a mixture of ethyl acetate and heptane 1:1 (60 ml) affording after drying in vacuo at 50
25 °C 1.2 g (52%) of the title compound as a solid.

m.p.: 197-199 °C

Calculated for $C_{12}H_8N_2O_2$:

C, 67.92 %; H, 3.80 %; N, 13.20 %. Found
30 C, 67.92 %; H, 3.73 %; N, 13.04 %.

EXAMPLE 5

5

5-(2-Naphthalen-2-yl-vinyl)-1,3,4-oxadiazole-2(3H)-thione

To a stirred solution of 2-naphthyl acrylic acid (5.0 g, 25.0 mmol) in dry tetrahydrofuran
10 (100 ml) and N,N-dimethylformamide (0.2 ml) was added dropwise at 0 °C oxalyl chlori-
de (4.8 g, 38.0 mmol). The mixture was stirred for 1 h at room temperature and the sol-
vent was evaporated in vacuo affording crude acid chloride. To a solution of tert-butyl
carbazate (6.7 g, 50.0 mmol) in dry tetrahydrofuran (80 ml) was added dropwise a solu-
15 tion of the acid chloride in dry tetrahydrofuran (50 ml) at 0 °C. The resulting reaction
mixture was stirred for 17 h at room temperature and the solvent was evaporated in
vacuo. To the residue was added water (200 ml) and ethyl acetate (200 ml) and the
phases were separated. The organic phase was washed with 0.1 N sodium hydroxide (2
x 100 ml), dried (MgSO₄), filtered and evaporated in vacuo. The solid residue was sus-
20 pended in heptane (50 ml), filtered off and dried in vacuo at 50 °C which afforded 4.7 g
(59%) of N-(3-naphthalen-2-yl-acryloyl)hydrazine carboxylic acid tert butyl ester as a so-
lid.

To a solution of the above hydrazine carboxylic acid tert butyl ester (4.5 g, 14.4 mmol) in
ethanol (25 ml) was added 2 N hydrochloric acid and the mixture was refluxed for 1 h.
25 The solvent was evaporated in vacuo and the residue was dissolved in water (100 ml)
and made alkaline to pH = 9 with 1 N sodium hydroxide. The precipitated was filtered off
and washed with water (2 x 30 ml) and heptane (2 x 30 ml) and dried in vacuo at 50 °C
which afforded 2.8 g (92%) of 3-naphthalen-2-yl-acrylic acid hydrazide as a solid.

To a stirred solution of the above acrylic acid hydrazide (1.5 g, 7.05 mmol) in absolute ethanol (15 ml) was added potassium hydroxide (0.40 g, 7.05 mmol) and carbondisulfide (1.50 g, 16.25 mmol) at 0 °C. After stirring for 1.5 h at 0 °C absolute ethanol (50 ml) was added and the reaction mixture was reflux temperature for 4 h. The reaction mixture was
5 evaporated in vacuo, and to the residue was added water (100 ml). The resultant mixture was acidified to pH = 1 with concentrated hydrochloric acid followed by addition of ethyl acetate (50 ml). The mixture was stirred for 0.5 h and the precipitate was filtered off, washed with water (2 x 10 ml) and diethyl ether (2 x 10 ml) and dried in vacuo at 50 °C which afforded 0.31 g (17%) of the title compound as a solid.

10

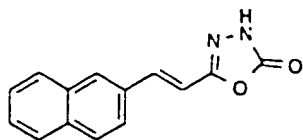
m.p.: > 250 °C

Calculated for $C_{14}H_{10}N_2OS \cdot x0.75 H_2O$

C, 60.09 %; H, 3.85 %; N, 10.01 %. Found

15

C, 60.06 %; H, 3.24 %; N, 9.85 %.

EXAMPLE 6

20

5-(2-Naphthalen-2-yl-vinyl)-1,3,4-oxadiazol-2(3H)-one

To a stirred suspension of 3-naphthalen-2-yl-acrylic acid hydrazide (1.0 g, 4.71 mmol,
25 prepared as described in example 5) and triethylamine (0.64 g, 6.28 mmol) in dry tetrahydrofuran (15 ml) was added carbonyl dimidazole (1.02 g, 6.28 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The resulting reaction mixture was evaporated in vacuo and to the residue was added water (25 ml). The precipitate was filtered off and washed with water (2 x 10 ml) and diethyl ether (2 x 10 ml) and re-

crystallised from ethyl acetate (100 ml) affording after drying in vacuo at 50 °C 0.5 g (45%) of the title compound as a solid.

m.p.: 252-254 °C

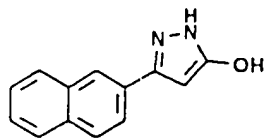
5 Calculated for $C_{14}H_{10}N_2O_2$:

C, 70.58 %; H, 4.23 %; N, 11.76 %. Found

C, 70.87 %; H, 4.22 %; N, 11.61 %.

EXAMPLE 7

10



5-Naphthalen-2-yl-2H-pyrazol-3-ol

15

To a solution of diethyl carbonate (18 ml) and sodium hydride (5.9 g, 0.15 mol, 60% dispersion in mineral oil) in dry toluene (60 ml) was added dropwise a solution of methyl 2-naphthyl ketone (10.0 g, 0.06 mol) in diethyl carbonate (7.4 g, 0.06 mol) and the resulting reaction mixture was slowly heated to 80 °C (exothermic) and diluted with toluene (50 ml, do to heavy precipitation). The reaction mixture was stirred and heated at 80 °C for 1 h. The cooled reaction mixture was quenched by carefully addition of water (100 ml) followed by addition of diethyl ether (100 ml). The phases were separated and the aqueous phase extracted with diethyl ether (100 ml). The combined organic phases were washed with water (100 ml) and saturated aqueous sodium chloride (100 ml), dried (MgSO₄), filtered and evaporated in vacuo. The residue (15 g) was purified by column chromatography on silicagel (900 ml) using a mixture of ethyl acetate and heptane (1:10) as eluent. This afforded 10.1 g (71%) of 3-(2-naphthyl)-3-oxo-propionic acid ethyl ester as an oil.

20

25

A mixture of the above β -keto ester (1.0 g, 4.13 mmol) and hydrazine hydrate (0.41 g, 8.25 mmol) in ethanol (15 ml) was stirred at reflux temperature for 17 h. The reaction mixture was cooled and the precipitate was filtered off and washed with ethanol (2 x 10 ml), dried in vacuo at 50 °C affording 0.3 g which was dissolved in a mixture of ethyl acetate (50 ml) and water (50 ml). 1 N hydrochloric acid was added to pH = 1 and the aqueous phase was separated off. The organic phase was washed with saturated aqueous sodium chloride (2 x 25 ml), dried (MgSO_4), filtered and evaporated in vacuo affording 75 mg (9%) of the title compound as a solid.

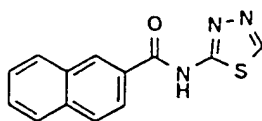
m.p.: 186 - 188 °C

Calculated for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O} \cdot x0.2 \text{ H}_2\text{O}$

C, 73.02 %; H, 4.90 %; N, 13.10 %. Found

C, 72.95 %; H, 4.78 %; N, 12.96 %.

EXAMPLE 8



Naphthalene-2-carboxylic acid [1,3,4]thiadiazol-2-yl amide

To a stirred solution of 2-naphthyl carboxylic acid (76.5 g, 0.45 mol) in dichloromethane (500 ml) was added thionyl chloride (38.7 ml, 0.53 mol) and the mixture was heated at reflux temperature for 48 h. The volatiles were evaporated in vacuo affording 80 g (94%) of 2-naphthoyl chloride.

To a stirred solution of 2-amino-1,3,4-thiadiazol (0.84 g, 8.0 mmol) in pyridine (5 ml) was added 2-naphthoyl chloride (1.9 g, 10 mmol). The resulting mixture was stirred at reflux temperature for 15 min. cooled and quenched with water (100 ml). The precipitate was

filtered off, washed with water (2 x 10 ml) and heptane (2 x 10 ml) and dried in vacuo affording 0.80 g (38%) of the title compound as a solid.

m.p.: 197 - 199 °C

5

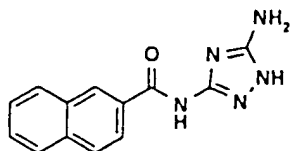
Calculated for $C_{13}H_9N_3OS$:

C, 61.16 %; H, 3.55 %; N, 16.46 %. Found

C, 61.49 %; H, 3.53 %; N, 16.52 %.

10

EXAMPLE 9



15 Naphthalene-2-carboxylic acid (5-amino-2H-[1,2,4]triazol-3-yl)-amide

A mixture of 2-naphthyl carboxylic acid (2.0 g, 11.6 mmol) and N,N'-carbonyldiimidazole (2.07 g, 12.8 mmol) in dry tetrahydrofuran (50 ml) was stirred at reflux temperature for 1
20 h. To the cooled reaction mixture was added 3,5-diamino-1,2,4-triazole (1.15 g, 11.6 mmol) and the resulting mixture was refluxed for 3 h. The cooled reaction mixture was quenched with water (75 ml) and extracted with diethyl ether (2 x 75 ml). The combined organic extracts were washed with saturated aqueous sodium chloride (2 x 50 ml), dried (MgSO₄), filtered and evaporated in vacuo. The residue was crystallised from diethyl et-
25 her (20 ml), filtered off and washed with diethyl ether (2 x 10 ml), dried in vacuo affor-
ded 0.95 g (32%) of the title compound as a solid.

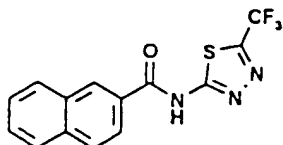
m.p.: 192 - 194 °C

30 Calculated for $C_{13}H_{11}N_5O$:

C, 61.65 %; H, 4.38 %; N, 27.65 %. Found

C, 61.96 %; H, 4.39 %; N, 27.11 %.

5 EXAMPLE 10



10 Naphthalene-2-carboxylic acid (5-trifluoromethyl-[1,3,4]thiadiazol-2-yl)-amide

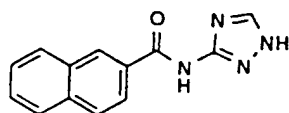
A mixture of 2-naphthyl carboxylic acid (2.0 g, 11.6 mmol) and N,N'-carbonyldiimidazole (2.07 g, 12.8 mmol) in dry tetrahydrofuran (50 ml) was stirred at reflux temperature for 1 h. To the cooled reaction mixture was added 2-amino-5-trifluoromethyl-1,2,4-thiadiazole (2.0 g, 11.6 mmol) and the resulting mixture was refluxed for 3 h. The cooled reaction mixture was quenched with water (100 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with water (2 x 50 ml), saturated aqueous sodium chloride (2 x 50 ml), dried (MgSO₄), filtered and evaporated in vacuo. The residue was crystallised from diethyl ether (20 ml), filtered off and washed with diethyl ether (2 x 15 ml) and heptane (2 x 15 ml), dried in vacuo at 50 °C afforded 1.4 g (37%) of the title compound as a solid.

m.p.: 249 - 251 °C

25 Calculated for C₁₄H₈N₃F₃OS:

C, 52.01 %; H, 2.49 %; N, 13.00 %. Found

C, 51.90 %; H, 2.40 %; N, 13.20 %.

EXAMPLE 11

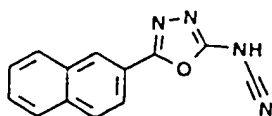
5

Naphthalene-2-carboxylic acid (4H-[1,2,4]triazol-3-yl)-amide

- 10 A mixture of 2-naphthyl carboxylic acid (3.0 g, 17 mmol) and N,N'-carbonyldiimidazole (3.1 g, 19 mmol) in dry tetrahydrofuran (75 ml) was stirred at reflux temperature for 1 h. To the cooled reaction mixture was added 3-amino-1,2,4-triazole (1.5 g, 17 mmol) and the resulting mixture was refluxed for 2.5 h. The cooled reaction mixture was quenched with water (150 ml) and the precipitate was filtered off and washed with water (2 x 20
- 15 ml), heptane (2 x 20 ml) and diethyl ether (2 x 20 ml), dried in vacuo at 50 °C affording 2.55 g (61%) of the title compound as a solid.

m.p.: 191 - 192 °C

- 20 Calculated for $C_{13}H_{10}N_4O$:
C, 65.54 %; H, 4.23 %; N, 23.52 %. Found
C, 65.30 %; H, 4.20 %; N, 23.61 %.

25 EXAMPLE 12

5-Naphthalen-2-yl-2,3-dihydro-[1,3,4]oxadiazol-2-yl-cyanamide

- 5 To a stirred solution of naphthalene-2-carboxylic acid hydrazide (0.7 g, 3.76 mmol) in isopropanol (40 ml) was added triethylamine (630 μ l, 4.51 mmol) and diphenyl cyanocarbonimidate (0.99 g, 4.14 mmol) and the resulting reaction mixture was stirred at room temperature for 1.5 h. The volatiles were evaporated in vacuo and to the residue was added water (50 ml) and diethyl ether (50 ml). The organic phase was separated and the
- 10 aqueous phase was acidified to pH = 1 with concentrated hydrochloric acid. The precipitate was filtered off and washed with water (3 x 10 ml) and diethyl ether (2 x 10 ml) and dried in vacuo at 50 °C which afforded 0.45 g (51%) of the title compound as a solid.

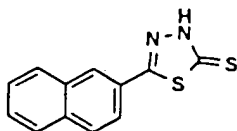
- 15 m.p.: > 250 °C

Calculated for $C_{13}H_{10}N_4O$:

C, 66.10 %; H, 3.41 %; N, 23.72 %. Found

C, 65.81 %; H, 3.33 %; N, 23.54 %.

20

EXAMPLE 13

- 25 5-Naphthalen-2-yl-3H-[1,3,4]thiadiazole-2-thione

- To a stirred solution of 1-naphthyl acrylic acid (20.0 g, 0.12 mol) in dry tetrahydrofuran (200 ml) and N,N-dimethylformamide (2 ml) was added dropwise at 0 °C oxalyl chloride
- 30 (21.5 ml, 0.256 mol). The mixture was stirred for 1 h at room temperature and the solvent was evaporated in vacuo affording crude acid chloride.

To a solution of 25% aqueous ammonium hydroxide (33 ml) in tetrahydrofuran (100 ml) was added dropwise a solution of the acid chloride in dichloromethane (150 ml) at 0 °C. The resulting reaction mixture was stirred for 1 h at room temperature. To the reaction mixture was added diethyl ether (200 ml) and water (200 ml). The precipitate was filtered
5 off and washed with water (2 x 75 ml) and diethyl ether (2 x 75 ml), dried in vacuo at 50 °C affording 20.0 g (100%) of naphthalene-2-carboxylic acid amide as a solid.

To a solution of the above amide (10.0 g, 58 mmol) in dry tetrahydrofuran (250 ml) was added [2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphenate-2,4-disulfide]
10 (Lawesson's reagent) (16.5 g, 41 mmol) and the resulting mixture was stirred at room temperature for 48 h. The volatiles were evaporated in vacuo and the residue was dissolved in ethyl acetate (100 ml) and filtered through silicagel (100 ml) using ethyl acetate as eluent. The solvent was evaporated in vacuo and to the residue was added a mixture of ethyl acetate (25 ml) and heptane (25 ml). The precipitate was filtered off and washed
15 with heptane (40 ml), dried in vacuo at 50 °C which afforded 9.0 g (83%) of naphthalene-2-carbothioic acid amide as a solid.

To a solution of the above carbothioic acid amide (8.0 g, 42 mmol) in methanol (200 ml) was added dropwise hydrazine monohydrate (3.3 ml, 68 mmol). The resulting reaction
20 mixture was stirred for 17 h at room temperature. The reaction mixture was evaporated in vacuo to 1/3 of its volume and purified by column chromatography on silicagel (1 l) using first ethyl acetate and later on a mixture of ethyl acetate and ethanol (1:1) as eluents. This afforded 4.4 g (56%) of naphthalene-2-carboximidic acid hydrazide as a solid.

25 To a solution of the above hydrazide (4.0 g, 22 mmol) in methanol (100 ml) was added dropwise carbon disulphide (3.4 ml, 56 mmol). The resulting reaction mixture was stirred for 4 h at room temperature. The precipitate was filtered off and washed with water (2 x 15 ml) and ethyl acetate (2 x 15 ml) and dried in vacuo at 50 °C yielding 1.7 g of crude
30 product which was recrystallised from ethyl acetate (20 ml) affording after drying in vacuo at 50 °C 1.3 g (85%) of the title compound as a solid.

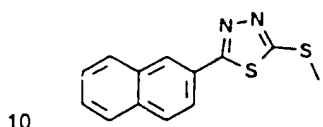
m.p.: 254 - 256 °C

Calculated for $C_{12}H_8N_2S_2$:

C, 58.99 %; H, 3.30 %; N, 11.47 %. Found

5 C, 59.10 %; H, 3.21 %; N, 11.35 %.

EXAMPLE 14



2-Methylsulfanyl-5-naphthalen-2-yl-[1,3,4]thiadiazole

15 To a solution of 5-naphthalen-2-yl-3H-[1,3,4]thiadiazole-2-thione (2.8 g, 11.5 mmol) in methanol (100 ml) was added dropwise 1 N sodium hydroxide (12 ml, 12 mmol) at 0 °C. After stirred for 10 min. at 0 °C iodomethane (2.0 g, 13.8 mmol) was added dropwise and stirring was continued at 0 °C for 5 min. and at room temperature for 2 h. The volatiles were evaporated in vacuo and to the residue was added water
20 (100 ml). The precipitate was filtered off and dried in vacuo at 50 °C. The dried compound (2.4 g) was recrystallised from ethyl acetate (60 ml) affording after drying in vacuo at 50 °C 0.7 g (23%) of the title compound as a solid.

m.p.: 133 - 135 °C

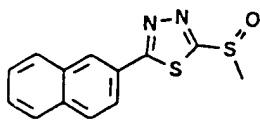
25

Calculated for $C_{13}H_{10}N_2S_2$:

C, 60.44 %; H, 3.90 %; N, 10.84 %. Found

C, 60.47 %; H, 3.89 %; N, 10.66 %.

30

EXAMPLE 15

5

2-Methanesulfinyl-5-naphthalen-2-yl-[1,3,4]thiadiazole

To a solution of 2-methylsulfonyl-5-naphthalen-2-yl-[1,3,4]thiadiazole
10 (1.3 g, 5.03 mmol) in dichloromethane (50 ml) was added 50% moisten 3-chloro peroxy-
benzoic acid (1.9 g, 5.53 mmol) at 0 °C. After stirred for 1 h at 0 °C the reaction was di-
luted with dichloromethane (50 ml) and quenched with addition of saturated aqueous
sodium hydrogen carbonate (50 ml). The organic phase was separated and washed with
water (50 ml), dried (MgSO₄), filtered and evaporated in vacuo. The residue (1.4 g) was
15 purified by column chromatography on silicagel (400 ml) using a mixture of ethyl acetate
and heptane (1:1) as eluent. This afforded after drying in vacuo at 50 °C 0.9 g (65%) of
the title compound as a solid.

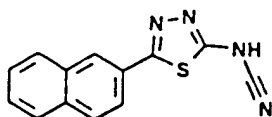
m.p.: 148 - 150 °C

20

Calculated for C₁₃H₁₀N₂OS₂:

C, 56.91 %; H, 3.67 %; N, 10.21 %. Found

C, 57.07 %; H, 3.71 %; N, 9.92 %.

25 EXAMPLE 16

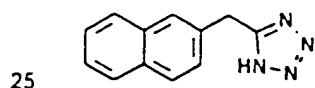
5-Naphthalen-2-yl-2,3-dihydro-[1,3,4]thiadiazol-2-yl-cyanamide

- 5 To a stirred solution of potassium tert-butoxide (0.46 g, 4.08 mmol) in tert-butanol (25 ml) was added cyanamide (0.32 g, 7.65 mmol) and the resulting mixture was stirred for 15 min. at room temperature. To this mixture was added 2-methanesulfinyl-5-naphthalen-2-yl-[1,3,4]thiadiazole (0.7 g, 2.55 mmol) and the mixture was heated at reflux temperature for 15 min. followed by addition of an additional portion of cyanamide
- 10 (0.2 g, 4.76 mmol). Heating was continued for an additional 2 h. The cooled reaction mixture was quenched by addition of 1 N sodium hydroxide (150 ml) and diluted by addition of diethyl ether. The organic phases were separated and the aqueous phase was acidified to pH = 1 with concentrated hydrochloric acid. The precipitate was filtered off, washed with water (2 x 10 ml) and diethyl ether (2 x 10 ml) and dried at 50 °C which
- 15 afforded 0.25 g (39%) of the title compound as a solid.

m.p.: > 260 °C

Calculated for $C_{13}H_{10}N_4S \cdot x0.25 H_2O$

- 20 C, 60.80 %; H, 3.34 %; N, 21.82 %. Found
C, 61.10 %; H, 3.07 %; N, 21.77 %.

EXAMPLE 175-(2-Naphthylmethyl)-1H-tetrazole

- 30 2-Bromomethylnaphthalene (5.00 g, 23 mmol) was dissolved in N,N'-dimethylformamide (50 ml) and potassium cyanide (2.95 g, 45 mmol) was added and the resulting mixture was stirred at room temperature for 16 hours. The supernatant was decanted and parti-

tioned between water (100 ml) and diethyl ether (2 x 75 ml). The combined organic phases were washed with water (100 ml), dried (MgSO_4), filtered and evaporated in vacuo affording 2.53 g (67%) of 2-naphtylacetonitrile as a solid.

5 m.p.: 84 - 85 °C.

$R_f = 0.12$ (SiO_2 : Ethyl acetate/heptane = 1:10)

A mixture of the above acetonitrile (2.50 g, 15 mmol), ammonium chloride (1.60 g, 30 mmol) and sodium azide (1.94 g, 30 mmol) in N,N' -dimethylformamide (25 ml) was stirred at 125 °C for 15 hours. The cooled reaction mixture was poured into water (300 ml) and acidified with 1 N hydrochloric acid, stirred at room temperature for 2 hours. The precipitate was filtered off and washed successively with water, a 1:1 mixture of diethyl ether and heptane and finally with heptane. The solid was dried by suction affording 1.69 g (53%) of the title compound as a solid.

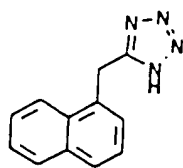
15

m.p.: 153 - 156 °C.

From the mother liquor further 1.05 g (33%) of the title compound was isolated giving a total yield of 86%.

20

EXAMPLE 13



25 5-(1-Naphtylmethyl)-1H-tetrazole

1-Chloromethylnaphthalene (5.00 ml, 33 mmol) was dissolved in N,N' -dimethylformamide (50 ml) and potassium cyanide (4.31 g, 66 mmol) and potassium iodide (0.1 g) were added and the resulting mixture was stirred at room temperature for 16

30

hours. The reaction mixture was partitioned between water (150 ml) and diethyl ether (2 x 100 ml). The combined organic phases were washed with water (100 ml), dried (MgSO_4), filtered and concentrated in vacuo affording 5.41 g (98%) of 1-naphtylacetonitrile as an oil.

5

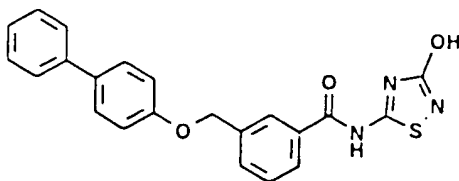
TLC: $R_f = 0.14$ (SiO_2 : Ethyl acetate/heptane = 1:10)

A mixture of the above acetonitrile (5.40 g, 32 mmol), ammonium chloride (2.59 g, 48 mmol) and sodium azide (3.15 g, 48 mmol) in *N,N'*-dimethylformamide (100 ml) was stirred at 125 °C for 16 hours. After cooling the mixture was poured into water (300 ml) and extracted with ethyl acetate (2 x 150 ml). The combined organic phases were washed with water (100 ml) and evaporated in vacuo. The residue was crystallised from diethyl ether (20 ml), filtered off and washed with diethyl ether affording 1.86 g (27%) of the title compound as a solid.

15

m.p.: 157 - 159 °C.

EXAMPLE 19



20

3-(Biphenyl-4-yloxymethyl)-N-(3-hydroxy-[1,2,4]thiadiazol-5-yl)-benzamide

25 To a stirred solution of 3-bromomethyl-benzoic acid methyl ester (30.9 g, 0.13 mol) and 4-phenylphenol (20.15, 0.12 mol) in dry *N,N'*-dimethylformamide (250 ml) was added potassium carbonate (48.1 g, 0.35 mol) and the resulting mixture was stirred for 20 h. The reaction mixture was poured on to water (600 ml) followed by addition of ethyl acetate (200 ml). The precipitate was filtered off and washed with water (2 x 50 ml) and

dried in vacuo at 50 °C which afforded 30.2 g (85%) of 3-(biphenyl-4-yloxymethyl)-benzoic acid methyl ester as a solid.

To the above benzoic acid methyl ester (12.3 g, 40.0 mmol) suspended in a mixture of
5 water (125 ml) and ethanol (125 ml) was added sodium hydroxide (4.80 g, 0.12 mol) and
the reaction mixture was heated at 60 °C for 20 h. The volatiles were evaporated in
vacuo and to the residue was added water (50 ml) followed by concentrated hydrochloric
acid to pH = 1. The resulting mixture was stirred at room temperature for 20 h and the
precipitate was filtered off and washed with water (3 x 25 ml), suspended in diethyl ether
10 (100 ml) and stirred for 2 h. The precipitate was filtered off and dried in vacuo at 50 °C
which afforded 10.23 g (81%) of 3-(biphenyl-4-yloxymethyl)benzoic acid as a solid.

To a mixture of the above benzoic acid (4.72 g, 15.0 mmol) and 2 drops of N,N'-
dimethylformamide in dry tetrahydrofuran (50 ml) was added dropwise thionyl chloride
15 (3.3 ml, 45 mmol) and the resulting reaction mixture was stirred at reflux temperature for
3 h. The volatiles were evaporated in vacuo and the solid residue was redissolved in dry
tetrahydrofuran (30 ml) and added dropwise to a solution of potassium thiocyanate (1.53
g, 15.3 mmol) in acetone (40 ml). The reaction mixture was stirred at room temperature
for 16 h, filtered and evaporated in vacuo. To a solution of the residue in acetone (50 ml)
20 was added urea (0.92 g, 15.3 mmol) and the resulting mixture was heated at reflux tem-
perature for 4 h. The cooled reaction mixture was evaporated in vacuo and the residue
was stirred for 0.5 h with ice water (100 ml). The precipitate was filtered off and washed
with water (2 x 25 ml), dried in vacuo at 50 °C. The crude product (6.03 g) was recrystal-
lised from acetonitrile (750 ml) affording 3.17 g (52%) of 3-(biphenyl-4-yloxymethyl)-N-
25 ureidocarbothioyl-benzamide as a solid.

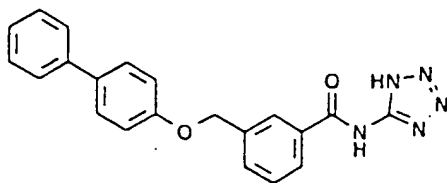
To a stirred solution of the above ureidocarbothioyl-benzamide (3.17 g, 7.8 mmol) in et-
hanol (30 ml) at 35 °C was added dropwise a 1 N solution of bromine in dichlorometha-
ne (7.8 ml, 7.8 mmol) during 10 min. The resulting reaction mixture was stirred for 0.5 h
30 at room temperature. The precipitate was filtered off, washed with diethyl ether (2 x 15
ml) and recrystallised from a mixture of N,N'-dimethylformamide and acetone (1:2) which

afforded after washing with acetone (10 ml) and diethyl ether (20 ml) and drying in vacuo at 50 °C 1.73 g (55%) of the title compound as a solid.

Calculated for $C_{22}H_{17}N_3O_3S$:

- 5 C, 65.49 %; H, 4.25 %; N, 10.42 %. Found
C, 65.40 %; H, 4.34 %; N, 10.10 %.

EXAMPLE 20



3-(Biphenyl-4-yloxymethyl)-N-(1H-tetrazol-5-yl)benzamide

- 15 To a mixture of 3-(biphenyl-4-yloxymethyl)benzoic acid (2.36 g, 7.5 mmol, prepared as described in example 20) and 2 drops of N,N'-dimethylformamide in dry tetrahydrofuran (25 ml) was added dropwise thionyl chloride (1.7 ml, 22.5 mmol) and the resulting reaction mixture was stirred at reflux temperature for 3 h. The volatiles were evaporated in vacuo and the solid residue was dissolved in dichloromethane (20 ml) and added
- 20 dropwise to a stirred suspension of 5-amino-tetrazole monohydrate (0.86 g, 8.3 mmol) and triethylamine (3.2 ml, 22.5 mmol) in dichloromethane (10 ml). After the addition was complete pyridine (5 ml) and 4-dimethylaminopyridine (10 mg) were added and the resulting mixture was stirred at room temperature for 48 h. The volatiles were evaporated in vacuo and the residue was suspended in water (100 ml) and acidified to pH = 3 with
- 25 concentrated hydrochloric acid. Ethyl acetate (100 ml) was added and the mixture was stirred for 0.5 h. The precipitate was filtered off and washed with water (2 x 10 ml) and dried in vacuo at 50 °C afforded 1.39 g (50%) of the title compound as a solid.

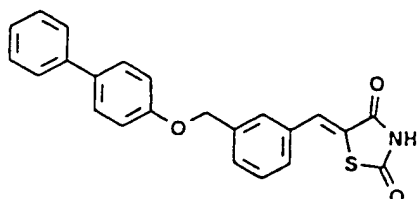
Calculated for $C_{21}H_{17}N_5O_2$: x0.1 triethylamine

C, 68.00 %; H, 4.89 %; N, 18.72 %. Found

C, 67.88 %; H, 4.68 %; N, 18.23 %.

EXAMPLE 21

5



5-(3-(Biphenyl-4-ylmethoxy)benzylidene)-2,4-thiazolidinedione

10

A mixture of 3-hydroxybenzaldehyde (6.02 g, 49 mmol), 4-phenyl-benzylchloride (10 g, 49 mmol) and potassium carbonate (20 g, 148 mmol) in N,N'-dimethylformamide (100 ml) was stirred at room temperature for 16 h. The mixture was poured into water (500 ml) and stirred for 1 h. The solid formed was filtered off, washed with water (2 x 200 ml) and heptane (2 x 75 ml) and dried in vacuo at 50 °C for 16 h affording 12.3 g (87%) of 3-(biphenyl-4-ylmethoxy)benzaldehyde as a solid. TLC showed presence of unchanged 4-phenylbenzylchloride.

15

TLC: R_f = 0.28 (SiO₂: Ethyl acetate/heptane = 1:10)

20

A mixture of the above benzaldehyde (5.00 g, 17 mmol), 2,4-thiazolidinedione (3.03 g, 26 mmol) and piperidine (0.35 ml, 3.5 mmol) in ethanol (75 ml) was stirred at reflux temperature for 16 h. The reaction mixture was cooled and the precipitated was filtered off and washed thoroughly with ethanol and dried in vacuo at 50 °C. The solid was first washed with a mixture of ethyl acetate, heptane and dichloromethane (1:1:6, 40 ml) and then washed with dichloromethane (20 ml). Drying in vacuo at 50 °C afforded 1.88 g (28%) of the title compound as a solid.

25

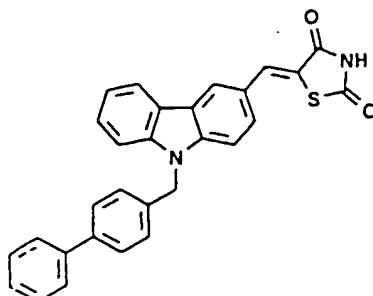
m.p.: 224 - 226 °C.

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EXAMPLE 22



- 5 5-((9-(4-Phenylbenzyl)-9H-carbazol-3-yl)-methylidene)-2,4-thiazolidinedione

- Carbazole (8.25 g, 49 mmol) was dissolved in N,N'-dimethylformamide (100 ml). Under a atmosphere of nitrogen was added sodium hydride (2.56 g, 64 mmol of a 60% suspen-
10 sion in mineral oil) in portion during 15 minutes. The mixture was then stirred at room temperature for 0.5 h. To the resulting mixture was added 4-phenylbenzylchloride (10.9 g, 49 mmol) in portions during 10 minutes. Then additional N,N'-dimethylformamide (100 ml) was added and the mixture was stirred at room temperature for 3.5 h. Water (125 ml) was added and the mixture was stirred vigorously at room temperature for 0.5 h, the
15 solid formed was filtered off, washed with water (2 x 100 ml) and with hexane (2 x 100 ml). Drying in vacuo at 50 °C for 16 h afforded 15.9 g (97%) of 9-(biphenyl-4-ylmethyl)-9H-carbazole as a solid.
TLC: R_f = 0.46 (SiO₂, ethyl acetate/heptane = 1:10).
20 Under a atmosphere of nitrogen at 0 °C phosphorous oxychloride (3.0 ml, 33 mmol) was added dropwise to N,N'-dimethylformamide (1.2 ml, 15.8 mmol). After the addition was complete, the mixture was stirred at 0 °C for 1 h and heated to 45 °C. At 45 °C the above carbazole (5.00 g, 15 mmol) was added during 15 minutes. The solid reaction mixture was then heated at 95 °C for 16 h. To the cooled reaction mixture water (125 ml) was
25 added and the mixture was stirred vigorously at room temperature for 4 h. The solid for- med was filtered off, washed with water and dried in vacuo at 50 °C affording almost quantitatively 9-(4-phenylbenzyl)-9H-carbazole-3-carboxaldehyde as a solid.

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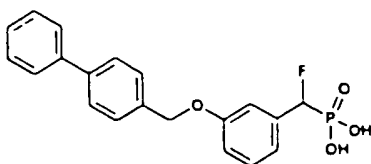
The above naphthalene (5.50 g, 17.6 mmol) was dissolved in toluene (50 ml), and diethylphosphite (2.50 ml, 19.4 mmol), triethylamine (2.9 ml, 21.1 mmol) and tetra-kis(triphenylphosphine)palladium(0) (1.02 g, 0.88 mmol) were added and the mixture was stirred at reflux temperature for 16 h. The mixture was concentrated in vacuo and the residue was purified by column chromatography on silica gel eluting with a mixture of ethyl acetate, heptane and triethylamine (50:50:1). This afforded 5.11 g (78%) of (3-(naphthalen-2-ylmethoxy)phenyl)phosphonic acid diethyl ester as a solid.

m.p.: 55 - 57 °C.

The above phosphonic acid diethyl ester (4.43 g, 12 mmol) was dissolved in acetonitrile (50 ml) and bromotrimethylsilane (3.5 ml, 26 mmol) was added. The resulting mixture was stirred at room temperature for 48 h. The slightly turbid mixture was filtered and the solvent evaporated in vacuo. The residue was dissolved in diethyl ether (100 ml) and methanol (6 ml) was added. The mixture was stirred at room temperature for 16 h and the precipitate filtered off, washed with diethyl ether and dried in vacuo at 50 °C for 16 h which afforded 3.51 g (93%) the title compound as a solid.

m.p.: 131 - 134 °C.

EXAMPLE 24



((3-(Biphenyl-4-ylmethoxy)-phenyl)fluoromethyl)phosphonic acid

3-(Biphenyl-4-ylmethoxy)benzaldehyde (5.0 g, 17 mmol, prepared as described in example 22) was mixed with di-tert-butyl phosphite (3.4 g, 17 mmol) and caesium fluoride (3.2 g, 21 mmol) was added and the mixture was stirred at room temperature for 5 h. The re-

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action mixture was diluted with dichloromethane (50 ml), filtered and concentrated in vacuo. The residue was crystallised from heptane, filtered and washed with heptane which afforded 7.20 g (86%) of ((3-(biphenyl-4-ylmethoxy)phenyl)hydroxymethyl)phosphonic acid di-tert-butyl ester as a solid.

TLC: $R_f = 0.24$ (SiO_2 , ethyl acetate/heptane = 1:1).

Diethylaminosulfur trifluoride (2.2 ml, 8.2 mmol) was dissolved in dichloromethane (25 ml) and the solution was cooled to -70°C and added dropwise to a solution of the above hydroxymethylphosphonic acid di-tert-butyl ester (4.0 g, 8.3 mmol) in dichloromethane (15 ml) at -70°C . The mixture was stirred at -70°C for 3 h and at room temperature for 20 h. With stirring the mixture was poured into 1 N aqueous potassium hydroxide (200 ml) and the mixture was extracted with dichloromethane (1 x 300 ml) and (1 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium chloride (100 ml), dried (MgSO_4), filtered and evaporated in vacuo affording 1.43 g (36%) of ((3-(biphenyl-4-ylmethoxy)-phenyl)fluoromethyl)phosphonic acid di-tert-butyl ester as a solid.

$^1\text{H-NMR}$ (200 MHz, CDCl_3): $\delta_H = 1.45$ (18H, d), 5.12 (2H, s), 5.5 (1H, dd), 6.95-7.65 (13H, m).

The above fluoromethylphosphonic acid di-tert-butyl ester (1.29 g, 2.7 mmol) was dissolved in dichloromethane (10 ml) and trifluoroacetic acid (2.5 ml) was added and the mixture was stirred at room temperature for 16 h. The mixture was filtered and the filtrate was evaporated in vacuo. The residue was partitioned between ethyl acetate (100 ml) and water (50 ml) and a solid was formed in the aqueous phase. This was filtered off and dried in vacuo at 50°C affording 55 mg (6%) of the title compound as a solid,

m.p.: 216°C (dec.).

EXAMPLE 25



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The PTP1B and PTP α cDNA was obtained by standard polymerase chain reaction technique using the Gene Amp Kit according to the manufacturer's instructions (Perkin Elmer/Cetus). The oligonucleotide primers were designed according to published sequences (Chernoff *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87: 2735-2739 (1990); Krueger *et al.*, *EMBO J.* 9: 3241-3252 (1990)) including convenient restriction nuclease sites to allow cloning into expression vectors. The cDNA corresponding to the full-length sequence of PTP1B and the intracellular part of PTP α were introduced into the insect cell expression vector pVL1392. The proteins were expressed according to standard procedures. PTP1B was semi-purified by ion exchange chromatography, and PTP α was purified to apparent homogeneity using a combination of ion exchange chromatography and gel filtration techniques using standard procedures. TC-PTP and LAR domain 1 were obtained from New England Biolabs. *Yersinia* PTP was a kind gift from J.E. Dixon, The University of Michigan, Ann Arbor, USA p-Nitrophenyl phosphate was purchased from Sigma and used without further purification.

15 Methods

p-Nitrophenyl phosphate (pNPP) is a general phosphatase substrate including a substrate for PTPases. When pNPP (colourless) is hydrolysed by a phosphatase to phosphate and p-nitrophenolate (yellow in alkaline solutions) the enzyme reaction can be followed by measuring the optical density at 410 nm after adjusting the pH appropriately. pNPP was used as general substrate to analyse the PTPase inhibitory capacity of the compounds of the invention.

The inhibiting effect of a compound is given by its K_i value, which expresses the concentration of inhibitor (μ M) in the reaction mixture necessary for a 50 percent reduction of the enzyme activity.

The K_i may be determined by a titration curve using several appropriately diluted solutions of the inhibitor or by using the following more simple formula, when the concentration of inhibitor is in large excess of the enzyme concentration:

$$K_i = I_0 \times E / (E_0 - E)$$

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where I_0 is the concentration of inhibitor (μM) added to the reaction mixture, E is the activity of the enzyme in the reaction mixture containing the inhibitor, and E_0 is the enzyme activity in a corresponding control reaction mixture without the inhibitor.

5

The K_i values of inhibitors towards PTP1B were measured as follows. In all cases the inhibiting effects were determined at pH 5.5 and at 37 °C with a reaction time of 60 minutes.

The reaction mixtures were:

10

- 1) 25 μl enzyme solution
25 μl inhibitor solution in DMSO
500 μl substrate solution

15 or

- 2) 25 μl enzyme solution
25 μl DMSO
500 μl substrate solution

20

The substrate solution contained 0.2 M acetate buffer, pH 5.5, 11 mM p-nitrophenyl phosphate, 5.5 mM dithiothreitol.

25

The reaction was stopped by addition of 4 ml 0.2 N NaOH, and the enzyme activity was determined by measuring the release of p-nitrophenol at 410 nm. The inhibiting effect was calculated as shown above.

30

The K_i values of inhibitors towards TC-PTP, LAR domain1, PTP α domain 1+2, and *Yersinia* PTP were measured essentially as described for PTP1B with the exception that all reactions were carried out in 96-wells microtiter plates. In all cases the inhibiting effects were determined at pH 5.5 and at room temperature with a reaction time of 15 minutes.

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63

The reaction mixtures were:

- 1) 5 µl enzyme solution
5 µl inhibitor solution in DMSO (final concentration 100 µM)
5 90 µl substrate solution
or
2) 5 µl enzyme solution
5 µl DMSO
90 µl substrate solution
10

The final concentrations: 0.2 M acetate buffer, pH 5.5, 5 mM p-nitrophenyl phosphate, 5 mM dithiotreitol.

- 15 The reaction was stopped by addition of 100 µl 0.4 N NaOH, and the enzyme activity was determined by measuring the release of p-nitrophenol at 405 nm. The inhibiting effect was calculated as shown above.

Results

- 20 Using the above assay systems we have demonstrated that compounds of the invention are PTPase inhibitors.

25





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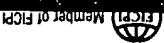
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Member of FICP

1. A compound of formula (I)

5 (L)_n—Ar₁—R₁—A

(I)

wherein

n is 1, 2, 3, 4, or 5 and (L)_n represents up to five (5) substituents which independently of each
10 other are hydrogen, C₁₋₆-alkyl, C₁₋₆-alkoxy, hydroxy, halogen, trihalogenomethyl, hydroxy-C₁₋₆-alkyl, amino-C₁₋₆-alkyl, -COR₂, -NO₂, -CN, -CHO, C₁₋₆-alkanoyloxy, carbamoyl, -NR₅R₆, aryloxy optionally substituted;

R₂ is C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted, -OH, -NR₃R₄ wherein
15 R₃ and R₄ independently of each other are hydrogen, C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted;

R₅ and R₆ are independently of each other hydrogen or C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted or -COZ₁ wherein Z₁ is C₁₋₆-alkyl, aryl optionally substituted,
20 aralkyl optionally substituted;

or L is A₁-Y₁-(W₁)-X-(W₂)-Y₂ wherein X is a chemical bond, -CO, -CONR₇, -NR₇CO, -NR₇, -O-, -S-, -SO, or -SO₂;

Y₁ and Y₂ are independently a chemical bond, -O-, -S-, or -NR₇;

25 R₇ is hydrogen, C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -COZ₂ wherein Z₂ is C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted;

30 W₁ and W₂ are independently a chemical bond or saturated or unsaturated C₁₋₆-alkylene;

A₁ is aryl optionally substituted, heteroaryl optionally substituted, biaryl optionally substituted, arylheteroaryl optionally substituted, -NR₈R₉ wherein R₈ and R₉ independently are hydrogen,

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C₁₋₉-alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -COZ₃ wherein Z₃ is C₁₋₉-alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted

or

5 when R₈ and R₉ together with the nitrogen atom forms a ring system A₁ is a saturated or partially saturated heterocyclic ring system optionally substituted with

C₁₋₉-alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -OH, C₁₋₉-alkoxy, hydroxy-C₁₋₉-alkyl, amino-C₁₋₉-alkyl, -COZ₄ wherein Z₄ is -OH, C₁₋₉-alkyl, NR₁₀R₁₁ wherein R₁₀ and R₁₁ independently are hydrogen, C₁₋₉-alkyl;

10

R₁ is a linker selected from a chemical bond, -C₁₋₉-alkyl-, -O(CH₂)_m-, -NR₁₂-, -CONR₁₂-, -NR₁₃CO-, -SO₂NR₁₄-, -NR₁₅SO₂-, -CR₁₆=CR₁₇-, -CH=, -CHR₁₇-, -CH₂-, -CHF-, -CF₂-, -SO₂-, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆ and R₁₇ are hydrogen, C₁₋₉-alkyl, aralkyl and m is 1, 2, or 3;

15

A is -PO(OR₁₈)(OR₁₉), -NH-SO₃H, -NH-SO₂-CH₃, -NH-SO₂-CF₃, -CO-NH-OH or a heterocycle as shown in scheme 1 wherein the point of attachment is indicated with a | (single bond) or || (double bond)

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optionally substituted by C_{1-6} -alkoxy, C_{1-6} -alkylthio; $-COOX^1$ wherein X^1 is C_{1-6} -alkyl optionally substituted by phenyl or benzyl optionally substituted;

R_{18} and R_{19} are C_{1-6} -alkyl, phenyl, benzyl;

R_{20} is hydrogen, $-OH$, C_{1-6} -alkoxy, $-SH$, C_{1-6} -alkylthio, $-COR_{21}$, $-SOR_{22}$, $-SO_2R_{23}$, $-NR_{24}R_{25}$,

5 $-NHCN$, halogen, trihalogenomethyl;

R_{21} , R_{22} and R_{23} are $-OR_{26}$, C_{1-6} -alkyl, $-NR_{24}R_{25}$, trihalogenomethyl;

R_{24} and R_{25} independently are hydrogen, C_{1-6} -alkyl, $-SO_2R_{26}$, $-COZ_5$ wherein Z_5 is

C_{1-6} -alkyl, trihalogenomethyl

R_{26} is hydrogen, C_{1-6} -alkyl, trihalogenomethyl;

10 nn is 1 or 2;

and Ar_1 is aryl or heteroaryl;

or a pharmaceutically acceptable salt thereof.

15 2. A compound according to the preceding claim: wherein

L is $A_1-Y_1-(W_1)-X-(W_2)-Y_2$ wherein X is a chemical bond, $-CO$, $-CONR_7$, $-NR_7CO$, $-NR_7$, $-O$, $-S$, $-SO$, or $-SO_2$;

Y_1 and Y_2 are independently a chemical bond, $-O$, $-S$, or $-NR_7$;

20 R_7 is hydrogen, C_{1-6} -alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, $-COZ_7$ wherein Z_7 is C_{1-6} -alkyl, aryl optionally substituted, aralkyl optionally substituted;

W_1 and W_2 are independently a chemical bond or saturated or unsaturated C_{1-6} -alkylene;

25

A_1 is aryl optionally substituted, heteroaryl optionally substituted, biaryl optionally substituted, arylheteroaryl optionally substituted, $-NR_8R_9$ wherein R_8 and R_9 independently are hydrogen, C_{1-6} -alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, $-COZ_3$ wherein Z_3 is C_{1-6} -alkyl, aryl optionally substituted, aralkyl optionally

30 substituted, heteroaryl optionally substituted

or

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when R₈ and R₉ together with the nitrogen atom forms a ring system A₁ is a saturated or partially saturated heterocyclic ring system optionally substituted with C₁₋₆-alkyl, aryl optionally substituted, aralkyl optionally substituted, heteroaryl optionally substituted, -OH, C₁₋₆-alkoxy, hydroxy-C₁₋₆-alkyl, amino-C₁₋₆-alkyl, -COZ₁ wherein Z₁ is -OH, C₁₋₆-alkyl, -NR₁₀R₁₁ wherein R₁₀ and R₁₁ independently are hydrogen, C₁₋₆-alkyl;

R₁ is a linker selected from a chemical bond, -C₁₋₆-alkyl-, -O(CH₂)_m-, -NR₁₂-, -CONR₁₂-, -NR₁₃CO-, -SO₂NR₁₄-, -NR₁₅SO₂-, -CR₁₆=CR₁₇-, -CH=, -CHR₁₇-, -CH₂-, -CHF-, -CF₂-, -SO₂-, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆ and R₁₇ are hydrogen, C₁₋₆-alkyl, aralkyl and

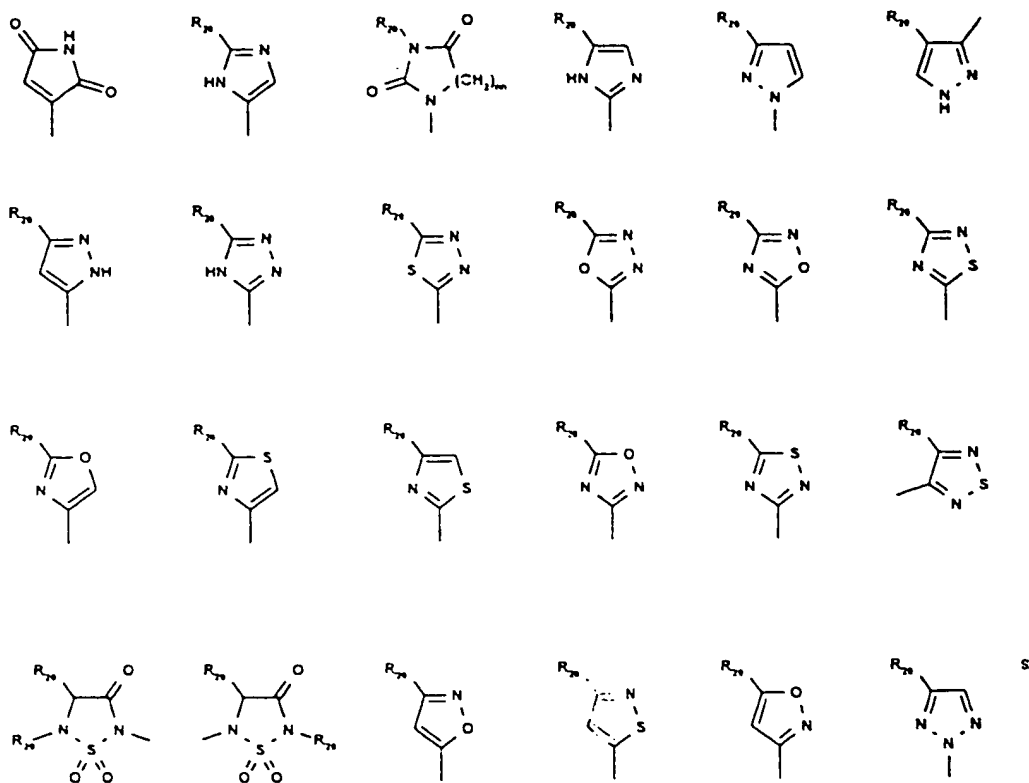
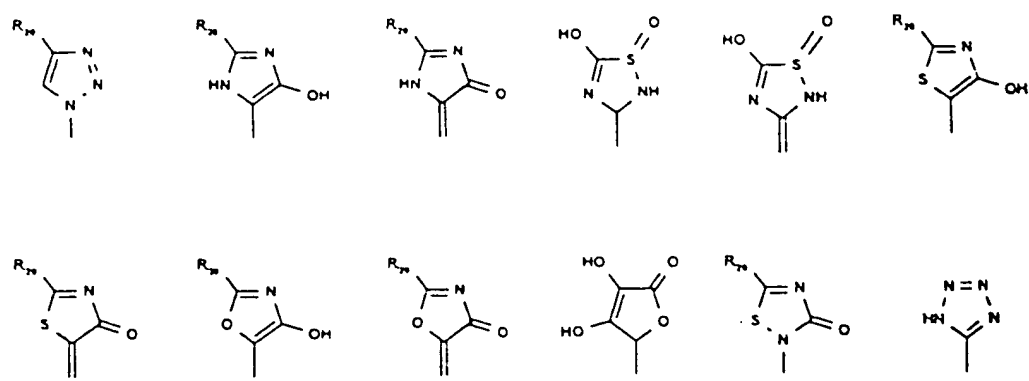
m is 1, 2, or 3;

A is -PO(OR₁₈)(OR₁₉), -NH-SO₃H, -NH-SO₂-CH₃, -NH-SO₂-CF₃, -CO-NH-OH or a heterocycle as shown in scheme 1 wherein the point of attachment is indicated with a (single bond) or || (double bond)

15

20

optionally substituted by hydrogen, C₁₋₅-alkyl optionally substituted by phenyl optionally substituted by C₁₋₅-alkoxy, C₁₋₅-alkylthio; -CCOX¹ wherein X¹ is C₁₋₅-alkyl optionally substituted by phenyl or benzyl optionally substituted;
R₁₈, and R₁₉ independently are hydrogen, C₁₋₅-alkyl, phenyl, benzyl;



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~~R₂₀ is hydrogen, -OH, C₁₋₆-alkoxy, -SH, C₁₋₆-alkylthio, -COR₂₁, -SOR₂₂, -SO₂R₂₃, -NR₂₄R₂₅,~~

-NHCN, halogen, trihalogenomethyl;

R₂₁, R₂₂, and R₂₃ are -OR₂₆, C₁₋₆-alkyl, -NR₂₄R₂₅, trihalogenomethyl;

R₂₄ and R₂₅ independently are hydrogen, C₁₋₆-alkyl, -SO₂R₂₆, -COZ₅ wherein Z₅ is

5 C₁₋₆-alkyl, trihalogenomethyl

R₂₆ is hydrogen, C₁₋₆-alkyl, trihalogenomethyl;

Ar₁ is aryl or heteroaryl;

nn is 1 or 2

and n is preferably 1, 2 or 3.

10

or a pharmaceutically acceptable salt thereof.

3. A compound according to any one of the preceding claims wherein Ar₁ is an optionally substituted phenyl, naphthyl or heteroaryl.

15

4. A compound according to any one of the preceding claims wherein R₁ is ethylene (-CH=CH-).

20

5. A compound according to claim 4 wherein the double bond configuration is trans (E).

6. A compound according to any one of the preceding claims wherein R₁ is -CONH- or -NHCO-.

25

7. A compound according to any one of the preceding claims wherein A is -NH-SO₃H, -NH-SO₂-CH₃ or -NH-SO₂-CF₃.

8. A compound according to any one of the claims 1-6 wherein A is -PO(OH)₂.

30

9. A compound according to any one of the preceding claims wherein A is:

2-Hydroxy-1H-imidazol-4(5)-yl;

4(5)-Hydroxy-1H-imidazol-2-yl;

3-Hydroxy-1H-pyrazol-1-yl;

3-Hydroxy-1H-pyrazol-3-yl;

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10. A compound according to any one of the claims 1-6: wherein R₁ is -CF₂- or -CHF- and A is selected from the following:

- 3-Hydroxy-1H-pyrazol-5-yl;
- 3-Hydroxy-4H-1,2,4-triazol-5-yl;
- 3-Hydroxy-1,2,4-oxadiazol-5-yl;
- 3-Hydroxy-1,2,4-thiadiazol-5-yl;
- 5 2-Hydroxy-oxazol-4-yl;
- 2-Hydroxy-thiazol-4-yl;
- 4-Hydroxy-thiazol-2-yl;
- 5-Hydroxy-1,2,4-oxadiazol-3-yl;
- 5-Hydroxy-1,2,4-thiadiazol-3-yl;
- 10 5-Hydroxy-1,2,5-thiadiazol-4-yl;
- 3-Hydroxy-isoxazol-5-yl;
- 3-Hydroxy-isothiazol-5-yl;
- 5-Hydroxy-isoxazol-3-yl;
- 1-Oxo-5-hydroxy-2,3-dihydro-1,2,4-thiadiazol-3-yl;
- 15 1-Oxo-5-hydroxy-2,3-dihydro-1,2,4-thiadiazol-3-ylidene;
- 4-Hydroxy-1,2,3-triazol-2-yl;
- 4-Hydroxy-1,2,3-triazol-1-yl;
- 2,4-Dihydroxy-imidazol-5-yl;
- 2-Hydroxy-4-oxo-imidazol-5-ylidene;
- 20 2,4-Dihydroxy-thiazol-5-yl;
- 2-Hydroxy-4-oxo-thiazol-5-ylidene;
- 2,4-Dihydroxy-oxazol-5-yl;
- 2-Hydroxy-4-oxo-oxazol-5-ylidene;
- 3-Oxo-5-hydroxy-1,2,4-thiazolidin-2-yl;
- 25 1,1-Dioxo-3-oxo-[1,2,5]thiadiazolidin-2-yl;
- 1,1-Dioxo-3-oxo-[1,2,5]thiadiazolidin-5-yl;
- 2-Oxo-3,4-dihydroxy-5H-furan-5-yl;
- 2-Oxo-4-hydroxy-5H-furan-3-yl;
- Tetrazol-5-yl;
- 30 2,5-Dioxo-pyrrol-3-yl;
- or tautomers thereof.

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2,4-Dihydroxy-thiazol-5-yl;

2,4-Dihydroxy-oxazol-5-yl.

11. A compound according to any one of the preceding claims selected from the following:
- 5 5-Naphthalen-2-yl-3H-[1,3,4]oxadiazole-2-thione;
Naphthalene-2-carboxylic acid (3-oxo-2,3-dihydro-[1,2,4]thiadiazol-5-yl)-amide;
5-Naphthalen-2-yl-[1,3,4]oxadiazol-2-ylamine;
5-Naphthalen-2-yl-3H-[1,3,4]oxadiazol-2-one;
- 10 5-(2-Naphthalen-2-yl-vinyl)-1,3,4-oxadiazole-2(3H)-thione;
5-(2-Naphthalen-2-yl-vinyl)-1,3,4-oxadiazol-2(3H)-one;
5-Naphthalen-2-yl-2H-pyrazol-3-ol;
Naphthalene-2-carboxylic acid [1,3,4]thiadiazol-2-yl amide;
Naphthalene-2-carboxylic acid (5-amino-2H-[1,2,4]triazol-3-yl)-amide;
- 15 Naphthalene-2-carboxylic acid (5-trifluoromethyl-[1,3,4]thiadiazol-2-yl)-amide;
Naphthalene-2-carboxylic acid (4H-[1,2,4]triazol-3-yl)-amide;
5-Naphthalen-2-yl-2,3-dihydro-[1,3,4]oxadiazol-2-yl-cyanamide;
5-Naphthalen-2-yl-3H-[1,3,4]thiadiazole-2-thione;
2-Methylsulfanyl-5-naphthalen-2-yl-[1,3,4]thiadiazole;
- 20 2-Methanesulfinyl-5-naphthalen-2-yl-[1,3,4]thiadiazole;
5-Naphthalen-2-yl-2,3-dihydro-[1,3,4]thiadiazol-2-yl-cyanamide;
Naphthalene-2-carboxylic acid (3-hydroxy-isoxazol-5-yl)-amide;
((3-(Biphenyl-4-ylmethoxy)-phenyl)fluoromethyl)phosphonic acid
N-(3-Hydroxy-(1,2,4-thiadiazol-5-yl)-4-methoxy-benzamide);
- 25 3-(Biphenyl-4-yloxymethyl)-N-(3-hydroxy-(1,2,4-thiadiazol-5-yl)benzamide;
9-Biphenyl-4-ylmethyl-9H-carbazol-3-carboxylic acid (3-hydroxy-(1,2,4-thiadiazol-5-yl)
amide;
5-(2-(3-(Biphenyl-4-yloxymethyl)-phenyl)vinyl)-1H-tetrazole;
2-(3-(Biphenyl-4-yloxymethyl)-phenyl)vinyl phosphonic acid;
- 30 5-(Difluoro-(4-(2-(methyl-pyridin-2-yl-amino)ethoxy)-phenyl)-methyl)-thiazolidine-2,4-dione;
5-(((4-(2-(5-Ethyl-pyridin-2-yl)-ethoxy)-phenyl)-difluoro-methyl)-thiazolidine-2,4-dione;
5-((2-benzyl-chroman-6-yl)-difluoro-methyl)-thiazolidine-2,4-dione;
5-(Difluoro-(4-(3-(5-methyl-2-phenyl-oxazol-4-yl)-propionyl)-phenyl)-methyl)-thiazolidine-2,4-
dione;



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5-(Difluoro-(4-(6-hydroxy-2-(5-methyl-2-phenyl-2-ethoxy)-phenyl)-methyl)-

thiazolidine-2,4-dione; or

5-(Difluoro-(4-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-phenyl)-methyl)-

thiazolidine-2,4-dione.

5 5-(2-Naphtylmethyl)-1H-tetrazole;

5-(1-Naphtylmethyl)-1H-tetrazole;

3-(Biphenyl-4-yloxymethyl)-N-(1H-tetrazol-5-yl)benzamide;

5-(3-(Biphenyl-4-ylmethoxy)benzylidene)-2,4-thiazolidinedione;

5-((9-(4-Phenylbenzyl)-9H-carbazol-3-yl)-methylidene)-2,4-thiazolidinedione;

10 (3-(Naphthalene-2-ylmethoxy)phenyl)phosphonic acid;

or a pharmaceutically acceptable salt thereof.

12. A method of preparing a compound according to any one of the preceding compounds claims, characterised in

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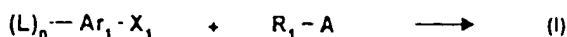


(II)

(III)

allowing a compound of formula (II) wherein $(L)_n$, Ar_1 , R_1 , and n are as defined above to react with an azide of formula (III) wherein M is defined as above in order to obtain the

20 compounds of formula (I) wherein A is a 5-substituted tetrazole; or

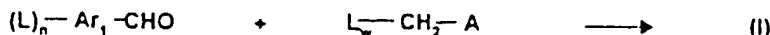


(VIII)

(IX)

allowing a compound of formula (VIII), wherein $(L)_n$, n , Ar_1 and X_1 are as defined above to react with a compound of formula (IX) wherein R_1 is $CH_2=CH$ and A is as defined above in order to obtain the compounds of formula (I); or

25



(X)

(XI)

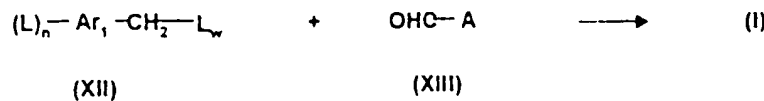
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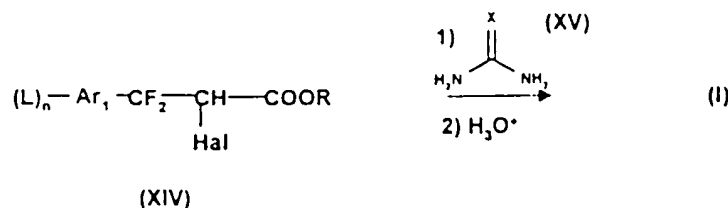
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allowing a compound of formula (X), wherein (L)_n and Ar₁ are as defined above to react

with a compound of formula (XI) wherein A and L_w are as defined above in order to obtain the compounds of formula (I); or



allowing a compound of formula (XII), wherein (L)_n, n, Ar₁ and L_w are as defined above to react with a compound of formula (XIII) wherein A is as defined above in order to obtain the compounds of formula (I); or



allowing a compound of formula (XIV), wherein (L)_n, n, and Ar₁ are as defined above and X is a suitably leaving group and R is C₁₋₆-alkyl to react with a compound of formula (XV) wherein X is O or S whereby a compound of formula (I) is produced wherein R₁ is CF₂ and A are 2,4-dihydroxy-oxazolidin-5-yl or 2,4-dihydroxy-thiazolidin-5-yl;

13. A pharmaceutical composition comprising as active component a compound according to any one of the preceding compound claims together with a pharmaceutically acceptable carrier or diluent.

14. A pharmaceutical composition suitable for modulating the activity of PTPases or other molecules with tyrosine phosphate recognition unit(s) comprising an effective amount of a compound according to any one of the preceding compound claims together with a pharmaceutically acceptable carrier or diluent.

15. The pharmaceutical composition according to any one of claims 13 or 14 suitable for treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance,

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~~insulin resistance, obesity, immune dysfunction's including autoimmunity and AIDS,~~

- diseases with dysfunction's of the coagulation system, allergic diseases, osteoporosis, proliferative disorders including cancer and psoriasis, diseases with decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of
- 5 hormones or cytokines that regulate the release of/or response to growth hormone, diseases of the brain including Alzheimer's disease and schizophrenia, and infectious diseases.
16. The pharmaceutical composition according to any one of the claims 13, 14 or 15 comprising between 0.5 mg and 1000 mg of a compound according to any one of the preceding compound claims per unit dose.
17. A method of modulating the activity of PTPases or other molecules with phosphotyrosine recognition unit(s) in a subject in need of such treatment comprising
- 15 administering to said subject an effective amount of a compound or composition according to any one of the preceding compound or composition claims.
18. The use of a compound according to any one of the preceding compound claims for preparing a medicament.
- 20 19. The use of a compound according to any one of the preceding compound claims for preparing a medicament for modulating the activity of PTPases or other molecules with tyrosine phosphate recognition unit(s).
- 25 20. The use of a compound according to any one of the preceding compound claims for preparing a medicament for treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, obesity, immune dysfunction's including autoimmunity and AIDS, diseases with dysfunction's of the coagulation system, allergic diseases, osteoporosis, proliferative disorders including cancer and psoriasis, diseases with
- 30 decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of hormones or cytokines that regulate the release of/or response to growth hormone, diseases of the brain including Alzheimer's disease and schizophrenia, and infectious diseases.

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21. The use of a compound according to any one of the preceding compound claims for preparing a medicament for treating a subject in need of such treatment.
22. The use of a compound according to any one of the preceding compound claims for preparing a medicament for use as an immunosuppressant.
23. An immobilised compound comprising a suitable solid-phase coupled with a compound according to any one of the preceding compound claims.
24. A method for coupling a compound according to any one of the preceding compound claims to a suitable solid-phase matrix.
25. A method for isolating a protein or a glycoprotein with affinity for a compound according to any one of the preceding compound claims from a biological sample, comprising
 - contacting an immobilised compound according to claim 23 with said biological sample in order for said immobilised compound to form a complex by binding said protein or glycoprotein
 - removing unbound material from said biological sample and isolating said complex
 - extracting said protein or glycoprotein from said complex.
26. A method for isolating a protein-tyrosine phosphatase with affinity for a compound according to any one of the preceding compound claims from a biological sample, comprising
 - contacting an immobilised compound according to claim 23 with said biological sample in order for said immobilised compound to form a complex by binding said protein-tyrosine phosphatase
 - removing unbound material from said biological sample and isolating said complex
 - extracting said protein-tyrosine phosphatase.
27. A method for isolating a Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein with affinity for a compound according to any one of the preceding compound claims from a biological sample, comprising

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32. A method for determining the function of a given protein-tyrosine phosphatase or group of protein-tyrosine phosphatases or said molecules with phosphotyrosine recognition unit(s) in a cell or a subject using a compound according to claim 28 comprising
- 5 - contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s) measuring the biological effects induced by said complex.